Neuropeptides derived from pro-opiocortin

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Neuropeptides Derived From Pro-Opiocortin: Behavioral, Physiological, and Neurochemical Effects

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I. INTRODUCTION

Hormones secreted by the pituitary and the hypothalamus play an important role in the behavioral adaptation of an organism to its environment. These hormones regulate homeostasis and create the conditions in which the animal can cope optimally with environmental demands. The concept that the brain serves as a target organ for these hormones stems from the finding that adaptive behavior is impaired after removal of the pituitary gland. Treatment with pituitary hormones such as adrenocorticotropic hormone (ACTH), melanocyte-stimulating hormone (MSH), or vasopressin restored this behavior (483). Fragments of these hormones that lack the classic endocrine effects [e.g., $ACTH_{4-10}$ and des-Gly^9-vasopressin (see Tables 1 and 2)] also relieved the behavioral deficits. Furthermore ACTH, MSH, or β-endorphin and related peptide hormones were shown to influence behavior in intact animals (483, 490). The effects were evident in a variety of behavioral tests and in various animal species and humans. The neuropeptides related to ACTH and α-MSH are involved in learning and memory, in analgesia, and in maintenance behavior. It has been suggested that these peptides increase motivation, attention, and arousal. The neurohypophyseal hormones affect memory processes as well; in addition influences on sleep, on drug-seeking behavior, and on the development of tolerance to, and dependence on, addictive drugs have been demonstrated (354).

It has been hypothesized that these behavioral effects are the result of a direct action on the central nervous system (CNS). These peptides were originally named neurogenic or neurotropic peptides, in view of these central effects. For convenience's sake, however, the term *neuropeptides* was chosen (495), which is now used to designate peptides affecting the nervous system and peptides present in neural tissue. The hypothesis that neuropeptides from anterior or posterior pituitary origin might influence behavior via a direct effect on the CNS has to explain how the pituitary manages to control brain function. For example, it is not clear whether the hormones from the anterior pituitary enter the CNS via the bloodstream or by retrograde transport from the pituitary. The question is whether the small amounts of peptide that might penetrate the blood-brain barrier once they are released into the bloodstream are sufficient to alter ongoing brain activity. A step toward resolving these questions has been the recent finding that peptides related to ACTH, MSH, and β-lipotropin (β-LPH) and their precursor peptides are not only present in the pituitary but in many brain structures as well. A

TABLE 1. *Amino acid sequences of several ACTH analogues*

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
$ACTH_{1-24}$	H-Ser-	Tyr-	Ser-	Met-	Glu-	His-	Phe-	Arg-	Trp-	Gly-	Lys-	Pro-	Val-	Gly-	Lys-	Lys-	Arg-	Arg-	Pro-	Val-	Lys-	Val-	Tyr-	Pro-OH
α-MSH	Ac-Ser-	Tyr-	Ser-	Met-	Glu-	His-	Phe-	Arg-	Trp-	Gly-	Lys-	Pro-	Val-OH											
$ACTH_{4-10}$	H-Met-	Glu-	His-	Phe-	Arg-	Trp-	Gly-OH																	
Org 2766	H-Met-(O_2)-	Glu-	His-	Phe-	Lys-	Phe-OH																		

TABLE 2. *Amino acid sequences of several endorphins*

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
β-Endorphin (β-LPH_{61-91})	H-Tyr-	Gly-	Gly-	Phe-	Met-	Thr-	Ser-	Glu-	Lys-	Ser-	Gln-	Thr-	Pro-	Leu-	Val-	Thr-	Leu-	Phe-	Lys-	Asn-	Ala-	Ile-	Val-	Lys-	Asn-	Ala-	His-	Lys-	Lys-	Gly-	Glu-OH
γ-Endorphin (β-LPH_{61-77})	H-Tyr-	Gly-	Gly-	Phe-	Met-	Thr-	Ser-	Glu-	Lys-	Ser-	Gln-	Thr-	Pro-	Leu-	Val-	Thr-	Leu-OH														
α-Endorphin (β-LPH_{61-76})	H-Tyr-	Gly-	Gly-	Phe-	Met-	Thr-	Ser-	Glu-	Lys-	Ser-	Gln-	Thr-	Pro-	Leu-	Val-	Thr-OH															
Met-enkephalin (β-LPH_{61-65})	H-Tyr-	Gly-	Gly-	Phe-	Met-OH																										
Leu-enkephalin	H-Tyr-	Gly-	Gly-	Phe-	Leu-OH																										

major breakthrough was the discovery that the brain contains endogenous peptides with opiatelike activity. After Hughes et al. (193) isolated and analyzed the two pentapeptides Met- and Leu-enkephalin, it was realized that the structure of Met-enkephalin is also present in β-LPH. This was the lead for the discovery of the opiatelike effects of the COOH-terminal part of this pituitary hormone. It was subsequently found that β-LPH and ACTH are derived from the same precursor molecule (283, 335) and that their release is controlled by the same mechanism (168). Immunohistochemical studies revealed that this precursor molecule exists in the brain (475). The nucleus arcuatus is the origin of that particular material (270). The precursor molecule, which has been named pro-opiocortin or pro-opiomelanocortin, is transported via peptidergic neurons to limbic midbrain structures (473). The primary structure of this 31,000-M_r precursor molecule has been proposed by Nakanishi et al. (317). It contains several pairs of basic amino acids, which on attack by proteolytic enzymes can yield various smaller peptides. Proteolytic cleavage of the COOH-terminal part of the pro-opiocortin molecule allows the formation of ACTH, β-LPH, and subsequently α-MSH, corticotropinlike intermediate lobe peptide (CLIP), γ-LPH, β-MSH, β-endorphin, and C′-fragment (β-LPH_{61-87}). Subsequent cleavage may be expected to yield additional peptides [e.g., γ-endorphin (β-LPH_{61-77}), des-Tyr^1-γ-endorphin (DTγE), α-endorphin (β-LPH_{61-76}), des-Tyr^1-α-endorphin (DTαE), and smaller fragments of β-endorphin] (67). The NH_2-terminal part of pro-opiocortin contains a structure more or less similar to that of α-MSH and it consequently has been termed γ-MSH (404).

The presence of the pro-opiocortin molecule in the brain and the fact that it can be cleaved to peptides with CNS effects give a clue about the mechanism of action of the behaviorally active neuropeptides. Conceivably pro-opiocortin, on release from neural cells, is degraded into multiple fragments that modulate complex brain functions underlying behavioral adaptation. Previous studies on effects of pituitary principles on behavior provided the basis for the notion put forward more than a decade ago that pituitary hormones function as precursor hormones for neuropeptides involved in CNS function (483). An important problem therefore still concerns the relative importance of neuropeptides from pituitary origin versus the brain-born neuropeptides. This review deals with this question. Nervous system effects of the neuropeptides derived from the pro-opiocortin molecule are reviewed with emphasis on ACTH; α-, β-, and γ-MSH; ACTH and MSH fragments; and β-, α-, and γ-endorphin and their respective fragments. Although Met-enkephalin comes from a different precursor molecule (33), its central effects are also described here because it is present in β-endorphin as the sequence β-LPH_{61-65}.

II. HYPOPHYSECTOMY AND ABNORMAL BEHAVIOR

Removal of the pituitary gland results in a number of behavioral deficits. Treated rats show an inferior learning performance, and nonconditioned

behavior is also impaired. It has been shown that hypophysectomy reduces the ability of rats to acquire a conditioned shuttle-box avoidance response (10, 11), although these rats do acquire the response when the interval between conditioned and unconditioned stimulus is longer (23). Subsequent experiments with a short (5-s) stimulus interval demonstrated that adenohypophysectomized animals are substantially inferior to their sham-operated controls in acquiring conditioned avoidance behavior (42, 480).

Impaired passive avoidance behavior has also been reported. Hypophysectomy attenuated the effect of a brief response-contingent shock of a bar-pressing response for glucose (8). Furthermore a one-trial passive avoidance response was impaired by hypophysectomy (479), and injection of ACTH restored the response. Similarly impaired one-trial passive avoidance behavior after weak or moderate shock has been reported by Lissák and Bohus (271). These authors found no effect of hypophysectomy after very high shock and suggested that fear motivation rather than learning is impaired in the absence of the pituitary.

Observations on conditioned behavior not motivated by fear are scarce and less conclusive. Stone and King (414) reported that hypophysectomy at an age of 40 days does not substantially affect learning in a relatively simple maze. Stone and Obias (415) subsequently found that rats hypophysectomized at the ages of 15, 30, and 35 days were significantly inferior to matched controls in a 13-choice swimming maze but only during the second half of the trial series. Rats hypophysectomized at 35 days of age and tested at a more adult age in a five-unit light-discrimination problem for a food reward were not different from sham-operated control animals. It was suggested that the behavioral disturbance in hypophysectomized rats is motivational.

Nonconditioned behavior has also been shown to change in response to hypophysectomy. Adenohypophysectomized rats had a lower running speed during escape from shock in a straight runway, and ACTH dose dependently normalized the performance (480). The ACTH fragment $ACTH_{4-10}$ failed to restore this behavior completely in totally hypophysectomized animals (482). Hypophysectomy did not affect self-stimulation in the lateral hypothalamus or ventral tegmentum (344) but reduced the increase in motor activity that was induced by morphine (227). Novelty-induced defecation was also reduced, and this latter effect was reversed by α-MSH. Although hypophysectomized rats and weight-matched controls were reported not to differ in a shock-responsiveness test (138), the behavioral response to electric footshock (flinch, jerk, and run) was elicited at lower shock intensities in hypophysectomized rats than in sham-operated controls. Treatment with $ACTH_{1-10}$ was not effective (150). This has been taken to indicate that a change in sensory perception does not underlie the impaired acquisition of avoidance behavior and the effect of ACTH. Furthermore the increase in exploratory behavior that is induced by hypophysectomy is not accompanied by a similar change in other behavioral categories such as maintenance behavior or play behavior (142). This suggests that the learning deficit of hypophysectomized rats is not caused by clumsy or disoriented behavior.

Because hypophysectomy results in multiple metabolic deficiencies, it is reasonable to suppose that the deficient avoidance behavior of hypophysectomized rats is caused by physical weakness. Indeed, extensive studies suggest that metabolic disturbances in hypophysectomized rats may play an important role in the impaired performance in active avoidance situations. Thus substitution therapy in adenohypophysectomized rats with thyroxine, cortisone, and testosterone normalized sensory and/or motor function studied in a straight runway under continuous shock punishment. Furthermore avoidance acquisition in a shuttle box was improved in these rats (480, 484). Treatment of hypophysectomized rats with either thyroxine or testosterone partially improved the avoidance behavior, perhaps because of the metabolic effect of these substances. Treatment with growth hormone also appeared to facilitate avoidance acquisition in these animals, but substitution therapy with a glucocorticosteroid was without effect (483, 484). A special diet that ensured good health of the hypophysectomized rat resulted in a similar improvement in conditioned avoidance behavior (173). Although important, however, the physical condition may not be the primary cause of the behavioral deficit of the hypophysectomized rats. This conclusion is derived from studies on passive avoidance behavior. This behavior is not associated with an increase but with a reduction in locomotor behavior and is attenuated in hypophysectomized rats (271, 479).

A deficiency in the pituitary-adrenal system is another factor that was originally considered the underlying cause of the behavioral impairment. Treatment with adrenal-maintenance doses of ACTH partly restored avoidance acquisition of adenohypophysectomized (480) and hypophysectomized rats (10, 479, 483). Although ACTH administration restored adrenocortical activity of hypophysectomized rats, the absence of corticosteroids cannot account for the behavioral deficiency, since avoidance behavior is not impaired by adrenalectomy (482). Furthermore treatment of hypophysectomized rats with the potent synthetic glucocorticosteroid dexamethasone failed to improve the acquisition of avoidance behavior (484). Accordingly the influence of ACTH on avoidance behavior of hypophysectomized rats seemed to be due to an extra-adrenal effect of the hormone. Experiments with peptides structurally related to ACTH but practically devoid of corticotropic activity substantiated this notion. Treatment of hypophysectomized rats with the ACTH fragments $ACTH_{1-10}$ or $ACTH_{4-10}$ or with α-MSH (i.e., *N*-acetyl-$ACTH_{1-13}$-amide) restored the acquisition of conditioned avoidance behavior (483). The behavioral effect of ACTH fragments cannot be due to a metabolic influence of these peptides, since $ACTH_{4-10}$ in amounts that restored avoidance learning failed to affect body growth, adrenal and testis weights, blood glucose, and the levels of insulin and free fatty acids in the plasma of hypophysectomized rats.

Peptides were originally administered as long-acting preparations given every other day during the training period. Consequently the administered peptides were continuously present due to the slow absorption of the peptide from a subcutaneous depot. They may therefore be expected to exert a tonic

influence on the CNS. In later experiments, however, administration of $ACTH_{4-10}$ as a short-acting preparation, dissolved in saline, prior to each daily session also resulted in normalization of shuttle-box avoidance acquisition in hypophysectomized rats (42).

III. PITUITARY VERSUS BRAIN-BORN NEUROPEPTIDES

The recent findings that ACTH, MSH, and related neuropeptides exist in the brain have questioned the notion that the pituitary is the sole or most important source of neuropeptides. Originally much research was directed at elucidating the mechanism whereby pituitary neuropeptides might gain access to the brain. Systemic administration of peptides in very low doses appeared to affect behavior and peptides do gain access to the brain (351), but the systemic circulation probably is not an efficient route. An alternative and more direct route is via the pituitary stalk or the liquor space around the gland. The direction of the blood flow in the stalk had been debated for many years (349, 513, 522) until it was settled by the brilliant work of Harris (161, 172). The greater part of the blood flows from the hypothalamus to the pituitary and thereby provides a means for the hypothalamic neurohormones to control pituitary function. More recently various workers have used more advanced techniques to unravel the fine structure of vascular connections in the pituitary vascular system. These studies suggested the possibility of retrograde blood flow toward the brain (25, 26, 337, 431, 444, 445). Such a retrograde flow (94, 300, 331) was the most likely explanation for the neuroendocrine short feedback (314) and for behavioral effects of pituitary peptides (483) and thus pointed to the pituitary as an important source of neuropeptides. This view, however, is challenged by the recent immunochemical evidence that there are distinct neuronal pathways in the brain containing various peptides that resemble those produced by the pituitary (33, 257, 474, 475). Furthermore ACTH and endorphins were produced from labeled amino acid precursors in tissue containing arcuate nucleus material (270), and the removal of the pituitary did not significantly affect the level of pituitary peptides in the brain (92, 247, 248, 375). This suggests that brain-born peptides may be involved in brain function.

The available evidence suggests that pituitary principles, in addition to the brain-born peptides, in some way are also involved in brain processes, because removal of the pituitary leads to marked behavioral disturbances. In addition administration of ACTH and ACTH fragments corrects these disturbances despite the presence of ACTH-like material in the brain. The generation of neuropeptides from brain-born precursor molecules may be disturbed in the absence of the pituitary. Some evidence in favor of this notion has recently been obtained. Bohus and de Wied (49) found that impaired shuttle-box avoidance training in hypophysectomized rats could be improved by peripheral administration of 8-arginine vasopressin (AVP) but

not by intracerebral injection of this neurohypophyseal principle. Similarly a combined hormone therapy with thyroxine, corticosterone, and testosterone normalized avoidance acquisition of hypophysectomized rats (484). In these animals intracerebroventricular (ICV) administration of vasopressin antiserum blocked the effect of the combined hormone therapy. The fact that the substitution therapy had a beneficial effect suggests that the generation of neuropeptides in the brain might have been restored.

As argued above, the physiological significance of pituitary hormones in adaptive behavior seems to be fairly well established. The physiological role of the brain-born peptides, however, is still unknown. For this reason an attempt was made to investigate the physiological importance of these brain-born neuropeptides in the acquisition and maintenance of active and passive avoidance behavior. Antisera to $ACTH_{1-24}$ and α-MSH were used to induce temporary bioinactivation of ACTH or α-MSH. In the first series of experiments, intact rats were used after they had completed the training of a pole-jumping avoidance response. Intracerebroventricular injection of ACTH antiserum prior to each daily extinction session facilitated extinction of the avoidance response, but α-MSH antiserum was inactive (509). Van Wimersma Greidanus et al. (509) mentioned the possibility that the ACTH antiserum might have bound some α-MSH, whereas the α-MSH antiserum only bound α-MSH. This could explain the finding that the combination of ACTH antiserum and α-MSH antiserum was most effective in facilitating extinction of the pole-jumping avoidance response. In another series of experiments, also performed with intact rats, a simple step through passive avoidance response was studied (1). Neither ACTH antiserum nor α-MSH antiserum nor the combination of the two affected the response when given immediately after the single learning trial, but the antiserums attenuated avoidance latency when given prior to the retention test.

Peptides related to ACTH have an interesting effect opposite to that of its antibody: these peptides delay extinction of pole-jumping avoidance behavior and facilitate passive avoidance behavior. A tentative conclusion from these experiments may be that brain-born peptides related to ACTH are involved in the maintenance of an avoidance response and are thus physiologically significant in adaptive behavior. However, more definite conclusions can be drawn only if similar experiments are done in hypophysectomized rats on replacement therapy, because these experiments were carried out in intact rats. The antiserums in these animals may therefore have inactivated ACTH and related peptides from pituitary origin as well as their brain-born peptides.

A point of concern is the puzzling finding that α-MSH antiserum was virtually inactive (509). The brain may process the pro-opiocortin molecule like the intermediate lobe of the pituitary (472) and thus produces α-MSH rather than ACTH (439). It is therefore possible that the ACTH antiserum also binds to a related but unknown neuropeptide that is more specifically involved in learning and memory. Some evidence in favor of such a conclusion

exists. For example, an antiserum to a potentiated $ACTH_{4-9}$ analogue (Org 2766) also facilitated extinction of pole-jumping avoidance behavior (274). This antiserum does not cross-react with any of the known peptides from the ACTH/MSH/endorphin family.

IV. ACTH-LIKE PEPTIDES AND CONDITIONED BEHAVIOR

A. Active Avoidance Behavior

Mirsky et al. (307) reported that the acquisition of an escape response in the rat was improved by ACTH. Murphy and Miller (316) subsequently found that rats treated with ACTH during acquisition of a shuttle-box avoidance response were more resistant to extinction; acquisition performance, however, was not affected. Treatment with ACTH also failed to affect acquisition of shuttle-box avoidance behavior in rats with posterior lobectomy. Again the rapid rate of extinction of the conditioned avoidance response that in these rats follows the removal of the posterior lobe of the pituitary was inhibited by the peptide (481). Because the extinction rate of an avoidance response is related to acquisition training, the effect of ACTH given during acquisition may be reflected in an improved performance during extinction.

Effects on acquisition performance have been obtained under certain conditions. Adrenocorticotropin facilitated the conditioned avoidance response during the early phase of conditioning, when the avoidance tendency is low (41). Similarly shuttle-box avoidance acquisition was facilitated by ACTH at low-shock-intensity punishment, when the tendency to respond is low. Administration of α-MSH also improved acquisition of shuttle-box avoidance behavior at a low level (but not at a high level) of shock (423). Facilitation of a Y-maze conditioned avoidance response by ACTH was demonstrated by Ley and Corson (265). The effect of the peptide treatment depended on the intensity of the shock and the activity of the rats, whereas the sex of the rats and the diurnal rhythm were additional important variables determining the behavioral effects of ACTH (266). During massed trials (which result in relatively slow acquisition), ACTH appeared to be effective in facilitating acquisition of a shuttle-box avoidance response. Male rats were used, and the effect was found only at the trough of the diurnal adrenal rhythm (336). In recent experiments with the same behavioral paradigm, $ACTH_{4-10}$ facilitated acquisition but [D-Phe^7]$ACTH_{4-10}$ had the reverse effect (50). In amygdalectomized rats ACTH also improved the low rate of acquisition of shuttle-box avoidance behavior (69). With a completely different experimental design, $ACTH_{1-10}$ was found to facilitate imprinting in ducklings (284). However, $ACTH_{1-24}$ had no such effect, whereas corticosterone attenuated imprinting. Panksepp et al. (338) observed facilitated avoidance acquisition in 1- and 3-day-old chicks without effects on general activity, distress vocalization, or tonic immobility. Habituation of an orienting

response to auditory stimuli in the rat was facilitated by $ACTH_{1-24}$ and $ACTH_{4-10}$ but not by [D-Phe^7]$ACTH_{4-10}$ (115).

Extinction of active avoidance behavior is more sensitive to the effect of neuropeptides than acquisition of that behavior. Although Murphy and Miller (316) found that administration of the peptide during the acquisition period delayed extinction, more pronounced effects of ACTH on both shuttle-box (482) and platform-jumping avoidance behavior (44) were observed when the peptide was administered throughout the extinction period.

The possibility that the effect of ACTH on extinction is not mediated through the adrenal cortex was first suggested by the findings that ACTH affected avoidance behavior in adrenalectomized rats as well as in intact rats (44, 305). In addition the heptapeptide $ACTH_{4-10}$, which in itself is devoid of corticotropic effects, in vivo was fully active on the avoidance behavior (483). The amino acid residue phenylalanine in position 7 plays a key role in the mediation of the behavioral effect of ACTH-like peptides: $ACTH_{1-10}$ delayed extinction of a shuttle-box conditioned avoidance response but its D-enantiomer [D-Phe^7]$ACTH_{1-10}$ facilitated extinction (45).

The influence of ACTH and related peptides is of a short-term nature. This conclusion emerged from studies in which the moment of peptide treatment appeared important. In one series of experiments, hypophysectomized rats received a daily injection of $ACTH_{4-10}$ 1 h prior to acquisition training in the shuttle box (42). The acquisition was improved, and when the peptide treatment was terminated after the 7th day of training, the treated rats displayed a high level of avoidance performance. Discontinuation of the treatment led to a progressive decrease in avoidance behavior despite continued punishment if the rat failed to respond to the conditioned stimulus. This effect was also found in another series of experiments with intact rats (44); discontinuation of ACTH administration during extinction sessions led to rapid extinction of the conditioned avoidance response (44). The delay of extinction apparently depended on the dose of peptide given. Administration of a single dose of $ACTH_{1-10}$ delayed extinction of pole-jumping avoidance behavior for 4–6 h (511), and the effect of higher amounts was of a somewhat longer duration. When high amounts of $ACTH_{4-10}$ were injected after the training, a delayed extinction of the active avoidance response was observed as long as 24 h after the injection. [D-Phe^7]$ACTH_{4-10}$ had the reverse effect (119). Miller et al. (302) failed to find an effect of $ACTH_{4-10}$ on a conditioned avoidance response in humans. The authors commented on the difference in species in relation to the task and also suggested that ceiling effects might have masked an effect. They favor the explanation that conditioned avoidance behavior is not a reliable measure for the behavioral effects of neuropeptides.

B. Passive Avoidance Behavior

The ACTH-like peptides facilitate the acquisition and retention of passive avoidance behavior. This has been demonstrated in several different

experimental situations. A low dose of ACTH given immediately after the learning of an inhibitory avoidance task in rats enhanced retention of the response, whereas a dose 10-fold higher had the reverse effect (151). The reverse effect of high doses of ACTH may be explained by an increased level of circulating corticosteroids that have effects opposite to those of ACTH (36, 504). When the interval between ACTH injection and learning trial was increased, no effect of the peptide on retention was found (152). These results suggest that ACTH affects memory-storage processes. The paradigm used by Levine and Jones (264) was the bar-press avoidance response for water after presentation of an electric shock. Administration of ACTH facilitated the acquisition of this response, and the treatment was more effective when given during both the acquisition and the retention period than when given during acquisition only. Facilitation of passive avoidance behavior was also observed in a three-stage thirst-versus-fear conflict situation (169). Treatment was effective when ACTH was given during approach training, avoidance training, or retention test but not when administered during both avoidance training and the retention test. Pappas and Gray (339) found that ACTH increases the latency to resume 200 licks for water in thirsty rats after electric shock within a single session. Lissák and Bohus (271) demonstrated that administration of $ACTH_{1-24}$ as a long-acting preparation prior to the single learning trial increases passive avoidance latencies in a situation where the innate dark preference of the rat was punished by electric footshock. The magnitude of the effect of the peptide appeared to depend on the intensity of the punishment. The lower the shock intensity, the more effective was the treatment. Administration of $ACTH_{1-24}$ and $ACTH_{4-10}$ also facilitated avoidance behavior in mice responding to attack by a dominant male (261, 373). Schneider et al. (391) measured the conditioned suppression of a drinking response and found that retention was improved when ACTH was given before training but not when administered before the retention test. The peptide had an effect only when the rats were conditioned in the morning. This is interesting, because the circadian cycle of pituitary-adrenal activity has a trough in the morning. Gray (159) found that ACTH facilitated passive avoidance behavior in a one-trial "step-out" learning situation when the peptide was administered during both training and testing. Rats that received ACTH during testing only, however, manifested decreased passive avoidance behavior. The author concluded that the effect of ACTH is state dependent. Facilitated passive avoidance behavior was also observed in a one-trial "step-through" learning situation. The peptides were given prior to the retention test. As in active avoidance situations, fragments of ACTH such as $ACTH_{1-10}$, $ACTH_{4-10}$, or $ACTH_{4-7}$ increased passive avoidance latencies (162). This was a short-term effect. Facilitation of passive avoidance behavior was observed after a single injection of ACTH-like peptides when these were given 1 h before the test session but not when administered immediately after the learning trial and tested 24 h later. Interestingly

[D-Phe^7]ACTH$_{4-10}$, which in active avoidance situations acts opposite to "all-L-peptides," had an effect similar to ACTH$_{4-10}$ in that it facilitated passive avoidance behavior (162). When the peptide was given in a high dose, however, passive avoidance behavior was impaired (119). Both peptides had to be administered within 1 h of training to be effective, and the behavioral effect of the D-peptide was of considerably longer duration.

Passive avoidance behavior of the rat has also been studied in experiments in which the aversive stimulus was not a footshock. A conditioned taste aversion for sugar water was induced by a lithium chloride injection, which makes the animal sick (369). Both ACTH$_{4-10}$ and [D-Phe^7]ACTH$_{4-10}$ (and also ACTH$_{1-24}$) delayed the extinction of the avoidance response. This finding was confirmed by Kendler et al. (233) and Hennessy et al. (181). These authors found that ACTH$_{1-24}$ was most effective when given prior to recovery trials. Administration of ACTH also potentiated the conditioned taste aversion induced by a subthreshold dose of morphine (405). Hennessy et al. (181) suggested that ACTH influences memory retrieval: the hormone may trigger a state-dependent phenomenon and thereby alter the retrieval process.

C. *Positively Reinforced Behavior*

Adrenocorticotropin and related peptides have been observed to influence rewarded behavior or approach behavior in a number of studies. However, it has recently been recognized that stimuli associated with approach behavior may be as stressful: the aversive quality may be as strong as in avoidance behavior. Observations by Levine et al. (263) demonstrated that any departure from expected schedules such as withdrawal of reward (extinction) or a reinforcement shift causes ACTH release. This means that uncertainty is potentially stressful. The stress may then account for the similarities in the effect of ACTH-like peptides in approach and avoidance situations and common mechanisms may therefore underlie their behavioral actions. Guth et al. (169) reported that ACTH increases the rate of a barpress response for water under conditions where the noise level and motivation were stringently controlled. Leonard (259) showed that ACTH administration partially antagonized the deleterious effect of sodium barbitone on running time for a food reward in a multiple T maze but failed to influence the effect of the drug on the errors made in the maze.

Although Hennessy et al. (180) did not observe an effect of ACTH on either acquisition or extinction of a food-reinforced runway response, effects on extinction performance in approach situations have been found. Gray et al. (158) studied the influence of ACTH on the acquisition and extinction of a runway response where food was used as the reward. Partial reinforcement resulted in an increased running speed during acquisition and in delayed extinction compared with the results of continuous reinforcement. A rela-

tively high dose of ACTH was administered during acquisition, and this treatment appeared to block the effect of partial reinforcement. When partially reinforced rats received ACTH, they behaved like continuously reinforced rats during both acquisition and extinction. In continuously reinforced rats, ACTH had no effect on performance. The extra-adrenal nature of this behavioral effect of ACTH has been demonstrated by Garrud et al. (131). Administration of $ACTH_{4-10}$ attenuated the alterations in response rate of bar pressing for food reward that normally occur when reward conditions change within a session. This peptide also attenuated the consequence of partial reinforcement on performance—i.e., resistance to extinction of a runway response for food reward—when it was administered during acquisition training (132). Isaacson et al. (195) trained water-deprived rats to approach one of four tables in an elevated X maze for water reward. They found that $ACTH_{4-10}$ improved the performance at the first trial.

Sandman, Kastin, and Schally (386) started a series of experiments in 1969 to explore the adaptive properties of α-MSH. Hungry rats were trained in a T maze for a food reward. Purified α- and β-MSH were administered for 5 consecutive days during acquisition, and delayed extinction of the appetitive response was found. In this particular experiment, however, the authors used impure preparations contaminated with vasopressin. The authors implied an effect of MSH on perseveration or memory and discarded a motivational hypothesis, since there was no effect on acquisition. However, because vasopressin has a powerful effect on extinction of avoidance behavior (481, 485, 488), the results of these studies may be attributed to the contaminating vasopressin. Stratton and Kastin (424) later found a facilitating effect of α-MSH on acquisition of an appetitive response in a 12-choice Warden maze; there was no effect on extinction of the task. Although Kastin et al. (223) found no effect of α-MSH on food and water intake, appetitive responses may be affected by a reduction in food intake and water consumption as a result of α-MSH treatment (338).

Adrenocorticotropin may directly influence reward mechanisms. Preliminary evidence in support of this notion was found in experiments in which rats were trained to press a lever to obtain $ACTH_{1-24}$ or $ACTH_{4-10}$ via an intrajugular catheter (221). Furthermore the involvement of opiate receptors, and consequently of the reward system, was suggested by the finding that naloxone inhibited the self-administration of ACTH. Colpaert et al. (84) in addition showed that $ACTH_{4-10}$ induces erroneous performance in rats trained to discriminate the synthetic opiate derivative fentanyl from saline. This discrimination requires the rat to attend to a complex set of stimuli. The $ACTH_{4-10}$ may have altered sensory input by an effect on attention. Self-stimulation was also sensitive to ACTH and related peptides. Nyakas et al. (325) found that subcutaneously administered $ACTH_{4-10}$ in rats enhanced bar pressing at low-intensity stimulation but not at currents higher than base line elicited from the medial septum and the medial forebrain bundle. The peptide lowered the threshold for self-stimulation but only in the medial

septum. At 3 times the threshold current, $ACTH_{4-10}$ decreased self-stimulation from the medial septum. Intracranial self-stimulation from the anterior medial forebrain bundle was also increased after administration of the $ACTH_{4-9}$ analogue (Org 2766) (228). These studies suggest that $ACTH_{4-10}$ may modulate the rewarding effect of brain stimulation.

D. Motivation, Learning, and Memory

Conclusions about the relevant mechanism of action of ACTH-like peptides have clearly been contradictory. On the one hand, it has been suggested that these substances have an influence on sensory, motivational, or attentional variables rather than on memory consolidation or retrieval. On the other hand, Flood and Jarvik (119) suggested that the ACTH-like peptides influence memory consolidation, and Sandman et al. (387) concluded that α-MSH affected memory or fear.

Adrenocorticotropin and related peptides facilitate acquisition, delay extinction of active avoidance behavior, delay extinction of rewarded behavior, and facilitate sexual motivation. These peptides also influence reward mechanisms. These findings were interpreted as an influence on motivation, a view supported by electrophysiological experiments and by studies on heart rate during passive avoidance behavior and in a classically conditioned emotional situation [for review, see Bohus and de Wied (49)].

Facilitated passive avoidance behavior in rats treated with $ACTH_{4-10}$ is accompanied by an increased heart rate compared with the heart rate before the learning trial. Analysis of the electrocardiographic changes during the entire passive avoidance period revealed that $ACTH_{4-10}$ treatment changed the distribution pattern of the interbeat (R-R) intervals, increasing the appearance of short R-R intervals. In contrast the distribution histogram in control rats showed a stepwise decline in the direction of longer R-R intervals. This may indicate that sympathetic influences on the heart rate, which normally are minimal, are facilitated by $ACTH_{4-10}$. The heart rate does not provide a simple index of the brain processes that underlie motivational or affective states. It has been suggested that the vagally mediated heart rate in a mildly stressful behavioral paradigm is related to attention and expectancy processes, whereas sympathetic influences are evoked by more intense stress in which the organism is actively engaged in the preparation and execution of activities to cope with the stress. Accordingly, increased sympathetic activity in $ACTH_{4-10}$-treated rats may reflect an increased state of arousal that increases the probability of a given behavioral performance in stressful situations. Experiments done under such conditions corroborate this view. Rats treated with $ACTH_{4-10}$ showed accelerated heart rate during extinction of a classically conditioned emotional response. Parallel to this generalized activation (arousal), a delay in extinction of the conditioned cardiac responses was found in peptide-treated rats. The effect on extinction

appeared to be associated with a heart rate deceleration. These findings indicate that ACTH-like peptides not only affect behavioral but also learned autonomic responses.

In the analysis of the behavioral effects of pituitary peptides, interest has also been focused on the relation with learning and memory processes. De Wied and Bohus (488) suggested that posterior pituitary principles, presumably vasopressin, influence long-term memory processes, and extensive work on vasopressin and vasopressin analogues substantiated this hypothesis (489). These long-term effects contrast with the short-term effects of α-MSH on avoidance behavior that may be due to enhancement of "trial-to-trial memory" (488). Several other interpretations have been proposed to describe the behavioral effects of MSH. Stratton and Kastin (422) used synthetic α-MSH in two T-maze tasks on shock-motivated behavior. No effect of the peptide was found on the performance of the first task, but when switched to the second task, rats treated with α-MSH took longer to learn that task. The rats were not further treated but received a second test 5 wk after the first, and the rats previously treated with α-MSH apparently had retained less than controls. The results were interpreted as an increase in motivational arousal that led to delayed extinction of the first task and slower learning of the second one. Improvement of attention was not implicated in these results, since α-MSH had no significant facilitatory effect on learning of either problem. However, an attentional hypothesis was subsequently proposed as the main behavioral effect of α-MSH and related peptides: this was derived from experiments with a discrimination paradigm (381, 382, 389). Rats were trained to avoid shock by running to a white door. After successful training, the task was reversed and running to the black door was the correct response. Administration of α-MSH appeared to have no effect on acquisition, but the treated rats required 50% fewer trials to solve the reversal-learning problem. In a recent study (385) the effects of various peptides related to ACTH, α-MSH, and β-MSH were tested; $ACTH_{4-10}$ and $ACTH_{1-24}$ improved learning of the original problem, but α-MSH, β-MSH_{1-18}, and β-MSH_{1-22} were inactive. Reversal learning, however, was facilitated by α- and β-MSH. Acquisition of the original response is considered a measure of learning a new response; the reversal stage then measures the attention level of the animal. According to the authors, these studies therefore suggest that MSH improves attentional processes and ACTH improves learning processes.

The influence of ACTH on memory processes has been examined in a number of experiments. The parameter studied was the amnesia induced by protein-synthesis inhibitors, anoxia, or electroconvulsive shock. Flexner and Flexner (118) reported that amnesia induced by intracerebral injection of puromycin in mice is prevented when ACTH (as a gel preparation) is administered up to 3 days before or within 16 h after learning. Subsequent experiments in which highly purified ACTH was used failed to replicate the former finding (255). It was suggested that the antiamnesic effect of the

ACTH preparation might have been due to vasopressin impurity, because this principle is highly effective in preventing amnesia (255). In these experiments the retention test was conducted 24 h after the original learning trial. When the interval between injection and trial was delayed (4 h), no effect on retention was measured. It was therefore concluded that ACTH acts on some residual (memory?) processes of the learning experience.

Administration of $ACTH_{1-24}$ also delays the onset of amnesia induced by cycloheximide or ouabain if given immediately after passive avoidance learning. The amnesia was reversed when a second injection of ACTH was given 1 h after the first injection (137). Both $ACTH_{1-24}$ and $ACTH_{4-10}$ had the same effect in KCl-induced amnesia. These observations suggest that ACTH and related peptides influence memory processes. Most of the observations favor the hypothesis that retrieval rather than storage of memory is affected by these peptides. Because vasopressin facilitates consolidation as well as retrieval processes (489), Bailey and Weiss (17) investigated the possibility that the memory effect of $ACTH_{4-10}$ is mediated by endogenous vasopressin in Brattleboro rats with diabetes insipidus. In these rats, which lack the ability to synthesize vasopressin, $ACTH_{4-10}$ apparently facilitated passive avoidance behavior; therefore the release of endogenous vasopressin does not mediate the effect of $ACTH_{4-10}$ on memory processes. This was confirmed by Bohus (38), who showed that high doses of $ACTH_{4-10}$ given after the learning trial corrected for the retention deficit in homozygous diabetes insipidus rats.

E. Sexual Behavior

Peptides related to ACTH also influence sexually motivated behavior. Bohus et al. (43) trained male rats to reach an estrogen-primed female in the goal box of a straight runway. On reaching the goal box, one group of male rats was always allowed to contact the female and achieve a complete ejaculation. Male rats in the control group could not contact the female because of a wire-mesh partition in the goal box. During extinction the female was removed from the goal box. Administration of $ACTH_{4-10}$ resulted in a delay of extinction of the runway response and in shorter running latencies, but only in those rats allowed to copulate during the training period.

Administration of $ACTH_{4-10}$ also influences the urge to seek contact with the incentive male in female rats (298). Ovariectomized female rats had to cross an electrified grid to gain contact with the male, but a copulatory response was prevented by a wire-mesh partition in the goal compartment. The grid current was then increased stepwise every second time the female crossed (increasing-barrier technique). Thus the animal had to endure a progressively aversive treatment to reach the goal box. Ovariectomized female rats made more crossing responses when they had received $ACTH_{4-10}$

on the 1st day of the training, but no effect was found when the peptide was administered during the trials.

The influence of ACTH and related peptides on male copulatory behavior has been studied extensively, but the data are rather controversial. Bertolini et al. (28, 29) reported that ICV administration of $ACTH_{1-24}$ or α-MSH elicits certain elements of the copulatory behavior pattern such as erection, ejaculation, sexual posturing, and genital licking in the male cat and rabbit even in the absence of a receptive female partner. These observations have been confirmed for $ACTH_{1-24}$ but not for $ACTH_{4-10}$ in rabbits (18, 174). MacLean (281) found that implantation of $ACTH_{1-24}$ or α-MSH in solid form in the septal-preoptic region results in penile erection in the squirrel monkey. Bertolini et al. (27) further reported that ICV injection of $ACTH_{1-24}$ shortens ejaculation latency in sexually experienced male rats. Lina and Bohus, however, in an attempt to replicate these observations, failed to find an effect of ICV-administered $ACTH_{1-24}$ on copulatory behavior of experienced male rats. However, the appearance of copulatory behavior was delayed by this peptide in inexperienced male rats (B. A. R. Lina and B. Bohus, unpublished observations). After systemic administration of a relatively crude ACTH preparation intromission and ejaculation frequency increased (408). In contrast, Korányi et al. (242) reported that intravenous administration of ACTH suppresses copulatory behavior of the male rabbit in a novel situation but not in the home cage. Repeated administration of $ACTH_{4-10}$ increased intromission and ejaculation latency in castrated male rats maintained on a threshold dose of testosterone. Replacement therapy with a larger dose of testosterone prevented this effect of $ACTH_{4-10}$ (43).

Systemic administration of $ACTH_{1-24}$ to ovariectomized estrogen-primed female rats induced lordosis behavior. This has been found to result from an increased progesterone output from the adrenals (110). Systemic administration of $ACTH_{4-10}$, which does not stimulate the adrenal cortex to produce progesterone, did not induce lordosis in ovariectomized estrogen-primed female rats. This ACTH fragment also failed to modify estrogen- and progesterone-induced lordosis behavior in these rats (298), but α-MSH had a biphasic effect in animals receiving submaximal doses of progesterone. Systemically administered α-MSH inhibited lordosis behavior in those rats showing high lordotic activity and stimulated lordosis behavior in those showing low lordotic activity. This was confirmed in a subsequent study (503). In addition, $ACTH_{4-10}$ was found to have the same effect, but $ACTH_{1-39}$ and $ACTH_{1-16}$ were inactive unless administered for 3 days. It was concluded that $ACTH_{4-10}$ contains the essential information for this effect. A similar biphasic effect was found for $ACTH_{4-10}$ in self-stimulation studies (324) and for ACTH in passive avoidance behavior (151). The biphasic effect of the peptide may be linked with the level of arousal of the animal, since an inverted U-shaped relationship exists between behavioral efficiency and arousal (197). Thus a peptide that induces arousal may either stimulate or inhibit lordosis depending on the animal's level of arousal (503). These ob-

servations indicate that ACTH and related peptides influence several aspects of male and female copulatory behavior. Such effects of ACTH, however, may not always represent increased sexual activity. For example, $ACTH_{1-24}$ induces penile erection and ejaculation, but such sexually "excited" male rabbits do not copulate with receptive female rabbits (29).

F. Structure-Activity Studies

Results obtained in pole-jumping active avoidance behavior showed a consistent dose-response relationship. Therefore structure-activity studies were performed to determine the regions in the ACTH molecule that are essential for the behavioral activity, i.e., the rate of extinction of pole-jumping avoidance behavior. Originally $ACTH_{4-10}$ appeared to be the shortest peptide with a potency comparable to that of the parent molecule (162), but subsequent studies showed that the tetrapeptide $ACTH_{4-7}$ is the shortest active sequence with essentially the same behavioral effect as ACTH (163).

As mentioned before, the phenylalanine in position 7 plays a key role in the mediation of the behavioral effect of the ACTH-like peptides. The compound [D-Phe^7]$ACTH_{1-10}$ (in which this amino acid was replaced by its D-enantiomer) appeared to facilitate rather than delay extinction of a shuttle-box avoidance response, and this effect has been found in intact as well as in hypophysectomized rats. The heptapeptide [D-Phe^7]$ACTH_{4-10}$ and the tetrapeptide [D-Phe^7]$ACTH_{4-7}$ appeared to be as active as the decapeptide in this respect. It is not clear what mechanisms underlie these opposite behavioral effects. Originally it seemed unlikely that the reversal effect could be due to competitive inhibition, because both the D- and the L-peptides facilitate passive avoidance behavior (486). (The only difference is that the effect of the D-peptides is of longer duration.) This suggested that the [D-Phe^7]ACTH analogues might contain an intrinsic new activity on extinction. Recent evidence, however, suggests that [D-Phe^7]$ACTH_{4-10}$ may antagonize endogenous ACTH-like neuropeptides: $ACTH_{1-24}$ but not $ACTH_{4-10}$ or $ACTH_{4-7}$ increased the firing rate of brain stem cells, and this response was markedly reduced by [D-Phe^7]$ACTH_{4-10}$. Responses to acetylcholine or L-glutamate were unaffected by the D-peptide (133). This specific and reversible antagonism may thus explain the opposite behavioral effects of D- and L-peptides.

The reversal of active avoidance behavior was found only for analogues with the phenylalanine residue in the D-configuration. Successive replacement of each of the other amino acid residues in the hexapeptide [Lys^8]-$ACTH_{4-9}$ by D-enantiomers failed to facilitate extinction of the avoidance response. Generally such D-enantiomer substitutions caused potentiation of the effect. This was strongest when lysine in position 8 was replaced by its D-enantiomer. This indicates that the structural requirements for behavioral and melanotropic activity are different, because it has been found (237, 525)

that in the sequence $ACTH_{6-10}$ melanotropic activity increased when the aromatic residues phenylalanine or tryptophan were replaced by their D-enantiomers. This activity was lost when the basic histidyl or arginyl residues were converted to their D-enantiomers.

Surprisingly the combination of D-methionine in position 4 with D-lysine in position 8 resulted in a decrease instead of an increase in potentiation. A similar combination of D-methionine in position 4 with D-phenylalanine in position 7 prevented the reversal of the behavioral effect caused by the introduction of the D-phenylalanine residue. Thus [D-Met^4, D-Phe^7]$ACTH_{4-10}$ appeared to delay extinction of a pole-jumping avoidance response. Obviously the configuration of the NH_2-terminal methionine, in itself not strictly essential, modulates the behavioral activity pattern in twofold-substituted analogues.

Substitution of phenylalanine by leucine in [Leu^7, Lys^8]$ACTH_{4-9}$ did not impair the behavioral activity and led to delay of extinction of the pole-jumping avoidance response. The effect was comparable to that found for $ACTH_{4-10}$. Substitution of phenylalanine by tryptophan in $ACTH_{4-10}$ slightly increased the potency, and substitution by pentamethylphenylalanine increased the potency even more. These results suggest that the electron-donor properties of the amino acid residue in position 7 may to some extent determine the behavioral potency.

The substitution of arginine by lysine in position 8, which is accompanied by loss of steroidogenic activity in [Lys^8]$ACTH_{1-24}$ (434), loss of steroidogenic and melanotropic activity in [Lys^8]$ACTH_{1-17}NH_2$, and loss of melanotropic activity in [Lys^8]$ACTH_{6-10}$ (79), did not reduce the behavioral activity. Replacement of tryptophan by phenylalanine in position 9 caused a marked decrease in steroidogenic activity in [Gln^5, Phe^9]$ACTH_{1-20}NH_2$ (188). This substitution did not damage the behavioral activity; even in the presence of [D-Lys^8], it induced a 100-fold increase. Oxidation of the S-CH_3 of the methionine molecule to the sulfone was another change in the molecule that decreased the steroidogenic activity of ACTH and the melanotropic activity of MSH (273) but increased the behavioral potency. The introduction of three of these modifications led to a 1,000-fold potentiation of extinction of pole-jumping avoidance behavior. Thus H-Met(O_2)-Glu-His-Phe-D-Lys-Phe-OH (Org 2766), when administered subcutaneously in nanogram quantities, appeared to inhibit extinction of pole-jumping avoidance behavior in intact rats (162). The same modifications led to a 1,000-fold decrease in melanotropic activity and also reduced the steroidogenic activity. In addition Org 2766 possessed neither fat-mobilizing effects nor opiatelike activity. The potentiating effect of Org 2766 was not observed in an inhibitory avoidance task. Martinez et al. (286) found an effect on retention of the response only if Org 2766 was administered 1 h before acquisition and not when injected 1 h before the retention test [as is seen with $ACTH_{4-10}$, which facilitates passive avoidance behavior when given prior to the retention test (486)]. Experiments by Fekete and de Wied (111), however, indicate that Org 2766 is also 1,000 times

more potent than $ACTH_{4-10}$ on passive avoidance behavior and effective both when given immediately after the learning trial and 1 h prior to the retention test.

A partial explanation for the effectiveness of the various substitutions on extinction of pole-jumping avoidance behavior may be found in their protection against enzymatic degradation. Incubation of ^{14}C-labeled $ACTH_{4-9}$ analogues with plasma or brain extracts revealed that the in vitro half-life of these analogues correlates with their behavioral potency (515). The peptide Phe-D-Lys-Phe appeared to be the main metabolite of the potentiated analogue, and all metabolic fragments contained less than 5% of the biological activity of Org 2766.

Although $ACTH_{4-7}$ is the shortest active fragment to have essentially the same behavioral potency as ACTH, more activity sites may be present. Early observations indicated that the sequence [D-Phe^7]$ACTH_{7-10}$ delayed extinction of a shuttle-box avoidance response (483). However, later experiments in the pole-jumping avoidance test with a newly synthesized peptide demonstrated facilitation of extinction. In both behavioral tests the effect appeared markedly less than that of [D-Phe^7]$ACTH_{4-10}$. The major breakdown product of the substituted hexapeptide Org 2766 (i.e., Phe-D-Lys-Phe) also possessed some behavioral activity. The same was true for $ACTH_{11-24}$, which delays extinction of pole-jumping avoidance behavior if given in sufficiently high quantities. In addition dogfish β-MSH was found to be as active as α-MSH (510). The common sequence H-His-Phe-Arg-Trp-OH in itself is less active than $ACTH_{4-10}$ on extinction of pole-jumping avoidance behavior (162). Eberle and Schwyzer (103) found that α-MSH also contains more than one activity site. Thus the essential requirements for the behavioral effect of ACTH analogues may not be restricted to a single locus or active core but may be present in at least two regions of the molecule, which show per se only marginal behavioral activity. This residual potency can be potentiated by chain elongation or by amino acid substitutions. Such modifications are believed to make the molecule more resistant to metabolic degradation or to induce a better fit on the putative receptor(s).

The residual behavioral potency observed for the sequence $ACTH_{7-10}$ could be increased to the same level as that of the reference peptide $ACTH_{4-10}$ by extending the COOH-terminal sequence to $ACTH_{7-16}$. A series of analogues of this peptide was synthesized. These analogues showed a steady increase in behavioral potency, culminating in a millionfold potentiation for the sequence: H-Met(O_2)-Ala-Ala-Phe-D-Lys-Phe-Gly-D-Lys-Pro-Val-Gly-Lys-Lys-NH_2 (Org 5042). Omission of either the glycyl residue in position 10 or a lysyl residue in position 16 was accompanied by a drastic decrease in potency. For high behavioral potency a doublet of basic lysine residues apparently is needed at exactly the same distance from the ACTH region 7-9 as is found in natural ACTH. This may be considered as an indication that the structural requirements for behavioral activity in the pole-jumping test are more related to ACTH than to MSH (163, 487). Some

arguments can be cited in favor of such a conclusion. *1*) The second affinity site becomes expressed after chain elongation to $ACTH_{7-16}$. *2*) The potency of modified ACTH fragments increases, since the analogue Org 2766 is behaviorally 1,000 times more active than $ACTH_{4-10}$ but possesses 1,000 times less MSH activity. *3*) Tryptophan in position 9 is essential for melanotropic activity but not for behavioral activity, since $ACTH_{1-8}$ has no melanotropic activity (188). *4*) Conversion of the basic amino acid in the 8-position to its D-enantiomer decreased the melanotropic activity of the peptide (237, 525), whereas the behavioral activity was increased.

G. Site of Action in the Brain

The research strategies used to explore the site(s) of action of pituitary-adrenal system hormones in the CNS have been diverse. In an attempt to block their behavioral effects, various brain regions have been destroyed. Implantation of ACTH-related peptides directly into specific brain regions has been another approach. Alternatively, specific receptor sites have been sought by binding experiments.

Bohus and de Wied (46) suggested that the thalamic parafascicular region is important in mediating the behavioral effect of ACTH-like peptides. Bilateral destruction of this area in the rat did not affect active avoidance acquisition, but this lesion resulted in facilitation of extinction. Administration of α-MSH failed to affect extinction of pole-jumping avoidance behavior in rats bearing lesions in the parafascicular nuclei (47). Subsequent experiments demonstrated that parafascicular lesions block the behavioral effect of $ACTH_{4-10}$ as well (506). More direct evidence of the involvement of the posterior thalamic area was obtained by implanting peptides in this region. Implantation of $ACTH_{1-10}$ delayed extinction but [D-Phe^7]$ACTH_{1-10}$ facilitated extinction of the avoidance response (511), as had been found after systemic administration. The parafascicular nuclei and the centrum medianum are parts of the nonspecific thalamic nuclei and play an important role in the maintenance of behavior. Lesions in this area impaired the retention of avoidance behavior (46, 71, 73, 88) and of instrumental avoidance and approach responses (3, 89) and caused retention deficits in visual learning (440). Electrical stimulation of the centrum medianum–parafascicular complex improved avoidance performance (72).

Although the implantation studies failed to demonstrate effective sites in the brain other than the parafascicular area, recent evidence suggests that the limbic forebrain may also be involved in the mediation of the behavioral effect of ACTH-like peptides. After bilateral destruction of the anterodorsal hippocampus, no effect of $ACTH_{4-10}$ was found on extinction of an active avoidance response (512). In addition systemic administration of ACTH normalized deficient shuttle-box acquisition behavior of rats bearing lesions in the amygdaloid nuclei (69), whereas lesions in the amygdaloid

nuclei blocked the effect of $ACTH_{4-10}$ on pole-jumping avoidance behavior (507). This was also found after transection of the fornix and the stria terminalis (508). It was suggested that the locus of action of ACTH and related peptides is not so much in a specific region of the brain but that the intact limbic system is needed for the expression of the neuropeptide effects.

The studies described above suggested that receptor sites for these neuropeptides might be present in limbic structures. Putative receptor sites for opiatelike peptides are present abundantly in the brain (254), but high-affinity binding sites for ACTH and related peptides have not yet been demonstrated in the brain (514). However, the multiplicity and specificity of ACTH-CNS interactions also point to the presence of specific ACTH receptors in the brain. Indeed the tritium-labeled highly potent $ACTH_{4-9}$ analogue Org 2766 is taken up preferentially in the septal area after intraventricular injection. In addition a significantly enhanced uptake of radioactivity in the septum was observed in hypophysectomized rats compared with that of control rats. The increased uptake in hypophysectomized rats could be prevented by chronic treatment with $ACTH_{1-24}$ or $ACTH_{4-10}$ given as a long-acting zinc phosphate preparation (459). Treatment with [D-Phe^7]$ACTH_{4-10}$, α-endorphin, or [des-glycinamide^9]lysine^8-vasopressin (DGLVP) did not affect the enhanced uptake of the $ACTH_{4-9}$ analogue in the septal region. Thus competitive displacement in vivo occurred only with peptides closely related structurally to NH_2-terminal ACTH fragments. Such studies suggest that receptor sites for ACTH fragments are present. The fact that such receptors have not yet been found may be explained by the high behavioral potency of modified peptides such as Org 2766. These receptors may therefore be characterized by high affinity and low capacity (514). They would represent an effector system that could effectively respond at very low neuropeptide concentrations. Interestingly, Rees et al. (362) have shown that after ICV administration radiolabeled Org 2766 appears intraneuronally in a small proportion of the cells near the cerebral ventricles (in the septum, caudate nucleus–putamen, preoptic area, hypothalamus, thalamus, amygdala, and hippocampus). An intracellular site of action could be possible, because transmembrane penetration of the NH_2-terminal part of $ACTH_{1-24}$ has been demonstrated in artificial lipid bilayer membranes (392).

Terenius (435) showed that $ACTH_{1-28}$ and $ACTH_{4-10}$ have appreciable affinity for stereospecific opiate-binding sites in rat brain synaptosomal plasma membranes. Structure-activity studies showed that the sequences $ACTH_{1-10}$, $ACTH_{4-10}$, $ACTH_{5-14}$, and $ACTH_{7-16}$ were active, whereas α-MSH, [Ac-Ser^1]$ACTH_{1-10}$, $ACTH_{4-9}$, $ACTH_{4-7}$, $ACTH_{5-10}$, and $ACTH_{7-10}$ did not have affinity for opiate-binding sites. These findings pointed to an active site in $ACTH_{4-10}$ with an additional site in the NH_2-terminal part of $ACTH_{11-24}$ (437). Analysis of the binding characteristics of $ACTH_{1-24}$ revealed a relatively low selectivity of the peptide for agonist or antagonist (436). The magnitude of the affinity constants of ACTH was of the order of 10^{-5}–10^{-6} M. A correlation exists between these in vitro studies and findings in vivo, since systemically

administered ACTH-like peptides in high doses reduced morphine analgesia in rats: only those ACTH fragments with affinity for opiate receptor sites inhibited morphine-induced analgesia (139). In addition $ACTH_{4-10}$ has been shown to antagonize morphine-induced behavioral activation after ICV administration in mice (226). Akil et al. (2) recently found that $ACTH_{1-24}$ also interacts with β-endorphin binding to rat brain membranes. Furthermore $ACTH_{1-24}$ discriminated between [^{3}H]β-endorphin- and [^{3}H]naloxone-binding sites, because it inhibited both high- and low-affinity binding components of β-endorphin but only the high-affinity binding of naloxone. These authors also commented on the physiological significance of this interaction because of the high concentration of $ACTH_{1-24}$ required for competition. Although it is unlikely that the receptor sites for β-endorphin and ACTH are identical, they may have similar characteristics, in view of the common biosynthetic origin of these peptides. A similar conclusion emerges from the work of Jacquet (203, 204). She suggested that there are two classes of opiate receptors in the periaqueductal region of rat brain. Although the term "ACTH receptor" has been questioned (6), Jacquet (204) proposed that the behavioral effects of opiates are mediated by these two receptors. One is a stereospecific opiate β-endorphin receptor mediating the analgesic and catatonic effects, and the second is a nonstereospecific opiate ACTH receptor mediating the stimulatory effects of opiates. In her experiments, high doses of the ACTH fragments (10–50 μg) locally injected into the periaqueductal gray induced a syndrome that resembled the abstinence signs of opiate withdrawal. Interestingly γ_2-MSH is more powerful in this respect. This peptide induced abstinence signs after direct application in the periaqueductal gray in much lower amounts than those needed for ACTH to elicit these symptoms (353).

V. EFFECTS OF ACTH AND ACTH FRAGMENTS ON GROOMING, STRETCHING, AND YAWNING

A. Stretching-and-Yawning Syndrome

Ferrari et al. (112, 113) showed that intracranial but not systemic injection of ACTH causes a behavioral stereotypy in mammals characterized by bouts of stretching and yawning. This stretching-and-yawning syndrome (SYS) was first described in dogs but could also be elicited in monkeys, rabbits, cats, guinea pigs, mice, and rats. It was demonstrated in adrenalectomized animals and was also elicited by α-MSH (136), indicating that it is an extra-adrenal effect. Both highly purified and synthetic α- and β-MSH produced the syndrome; β-MSH was more potent than α-MSH (113). The peptides $ACTH_{4-10}$, $ACTH_{5-10}$, and $ACTH_{6-10}$ were each increasingly less effective. These observations suggested that only part of the ACTH molecule was necessary for this neurotropic activity and that the sequence $ACTH_{4-10}$ was particularly important. The effect could be antagonized by chlorprom-

azine, atropine, and morphine but not by reserpine, lysergic acid diethylamide (LSD-25), γ-aminobutyrate (GABA), or monoamine oxidase inhibitors (136). The brain regions sensitive to ACTH were the hypothalamic areas lining the third ventricle, and the syndrome was accompanied by signs of electrophysiological and behavioral arousal. Huston (194) showed that yawning and penile erection in the rat can be brought about by cortical spreading depression and that $ACTH_{1-24}$ applied to the neocortex or the hippocampus resulted in spreading depression (206). Interestingly activation of central dopaminergic systems induces yawning in the rat. This can be blocked by dopamine receptor blockade (308). Urbá-Holmgren et al. (450), however, reported that yawning in the rat involves a central cholinergic mechanism with "muscarinic" receptors. In addition Wood et al. (520) found that intracranial α-MSH or $ACTH_{1-24}$ induced a twofold elevation of acetylcholine turnover in the hippocampus but not in any other structure. This suggests that a septal-hippocampal-cholinergic pathway is necessary to elicit SYS. The absence of this syndrome after total hippocampectomy is in line with this view (81).

Some authors found that intraventricular administration of ACTH induces sexual arousal in addition to SYS (18, 27–29, 136, 174). Evidence for a permissive effect of gonadal steroids in this response has been obtained (27). Haun and Haltmeyer (174) showed an increase in plasma testosterone in male rabbits and in plasma progesterone in females after intracranial ACTH. Intraventricular administration of $ACTH_{1-24}$ in guinea pigs induced SYS in castrated males in a dose-dependent manner. The syndrome, however, was markedly facilitated by daily subcutaneous injection of testosterone propionate [Rodriguez-Sierra et al. (374)]. Testosterone alone stimulated yawning. In contrast to other species, no excessive grooming, scratching, or wet-dog shaking was seen in the guinea pig in response to $ACTH_{1-24}$. In rats, however, the onset of SYS is preceded by the display of excessive grooming behavior (148, 201).

B. Excessive Grooming Behavior

It has long been known that birds and small mammals display enhanced grooming behavior in situations in which novel or conflicting environmental stimuli are present (51, 403, 441). Of course these same stimuli are known to activate the pituitary-adrenal system (287), implying that this system is involved in grooming induction. Because hypophysectomized rats still show novelty-induced grooming (215), however, the pituitary gland cannot be directly involved. Two lines of evidence suggest a role for centrally active ACTH in the induction of grooming. First, ICV administration of antibodies to ACTH reduced novelty-induced grooming (99). Second, intraventricular injection of ACTH or its NH_2-terminal fragments produced an enhanced display of grooming (148, 174, 201, 500). In view of the short latency of ACTH

administered by ICV injection and since its effects are independent of the adrenal cortex (148), this supports a direct CNS effect for ACTH in inducing excessive grooming. Furthermore systemic administration of the peptide failed to induce grooming, once again implying a direct CNS effect.

It was found that ACTH enhances the display of grooming behavior without changing the composition of the various components of grooming, consisting of head washing, body grooming, genital grooming, scratching, and licking. Thus ACTH did not produce more grooming episodes but lengthened their duration. The peptide-induced behavior was not a stereotypy and appeared unaffected by environmental factors: as much grooming was found in an enriched environment as in a stimulation-restricted test chamber (215). Only very strong motivational variables such as severe thirst or hunger and anxiety were able to modulate the ACTH-induced excessive grooming to a certain extent (216).

Novelty-induced grooming has been thought to reflect the influence of ACTH released from the pituitary (99). Although Dunn et al. (99) reported that hypophysectomy reduced novelty-induced grooming, Jolles et al. (215) found that hypophysectomized rats still showed novelty-induced grooming. This discrepancy may be the result of strain and age differences of the rats and differences in the pretreatment of the animals (140). It has been suggested that excessive grooming is a secondary response, dearousing the organism after its activation by ACTH either after exogenous administration or endogenous release (216).

Colbern et al. (82) found that injections of $ACTH_{1-24}$ given 24 h apart over several days produced comparable amounts of grooming. Jolles et al. (217), however, observed that a second administration of the peptide failed to induce excessive grooming when the second injection was within 8 h after the first one. This reduction in effectiveness was not due to fatigue or to "habituation to the experimental situation," because anesthesia during the first injection did not prevent the reduction in grooming of a second injection of $ACTH_{1-24}$ given 4 h later. Cross tolerance to this effect was found with β-endorphin, morphine, and [D-Phe^7]$ACTH_{4-10}$. Higher doses of $ACTH_{1-24}$ at the second administration restored the grooming response. Pretreatment of the animal with haloperidol or naloxone (which blocks the grooming response to ACTH) also prevented the development of acute tolerance (140, 217). According to the authors, ACTH may affect dopaminergic nigrostriatal and/or nigroaccumbens pathways that are involved in the grooming response. A temporary hyposensitivity of the dopamine receptors for a second injection of $ACTH_{1-24}$ may thereby be induced. Interestingly microinjection of haloperidol into the neostriatum but not into the substantia nigra suppressed ACTH-induced excessive grooming. Furthermore local injection of $ACTH_{1-24}$ into the substantia nigra caused excessive grooming, whereas the neostriatum and the accumbens area were not sensitive to $ACTH_{1-24}$ in this respect (85, 497). Suppression of ACTH-induced grooming was also found after injection of apomorphine or haloperidol in the neostriatum or apomorphine in the nucleus accumbens (85).

C. *Structure-Activity Studies*

In a series of experiments the effect of gradual shortening of the $ACTH_{1-24}$ sequence on the capacity to induce excessive grooming was studied. All peptides were tested at doses equimolar to 3 $\mu g/\mu l$ $ACTH_{1-24}$ (148). At this dose level the peptides $ACTH_{1-16}NH_2$, α-MSH, and β-MSH were as active as the sequence $ACTH_{1-24}$. The peptides [Lys^{17}-Lys^{18}]$ACTH_{5-18}NH_2$, $ACTH_{5-16}NH_2$, $ACTH_{1-13}NH_2$, and $ACTH_{5-14}$ also induced grooming but were less potent. The sequences $ACTH_{11-24}$, $ACTH_{7-16}NH_2$, $ACTH_{1-10}$, and (Ac-Ser^1)$ACTH_{1-10}$ were inactive (148). The fact that $ACTH_{1-24}$, α-MSH, and β-MSH were effective was ascribed to the common presence of the peptide $ACTH_{4-10}$. Surprisingly γ_2-MSH, which contains the sequence Met-Gly-His-Phe-Arg (317), did not induce excessive grooming in the rat (353).

Ferrari et al. (113), who selected the dog for their studies as being the most susceptible animal for central actions of ACTH, reported that high doses of $ACTH_{4-10}$ induced SYS. Baldwin et al. (18), reported that $ACTH_{4-10}$ has only marginal effects on SYS and sexual behavior after ICV administration into the rabbit. In the rat intraventricular $ACTH_{4-10}$ even in high doses did not induce excessive grooming (148, 496, 500). This finding is supported by observations of Rees et al. (361) in which $ACTH_{4-10}$ had no effect on excessive grooming in mice. Wiegant and Gispen (500) further found that, like $ACTH_{4-10}$, the sequences $ACTH_{4-9}$, $ACTH_{4-8}$, $ACTH_{4-6}$, $ACTH_{5-7}$, and $ACTH_{7-10}$ were inactive. The only short sequence that induced grooming was the sequence $ACTH_{4-7}$. On an equimolar basis this peptide had 30% of the activity of $ACTH_{1-24}$.

Studies with analogues of the $ACTH_{4-10}$ peptide also pointed to latent activity within the $ACTH_{4-10}$ sequence. [D-Phe^7]$ACTH_{4-10}$ possessed 50–60% of the activity of $ACTH_{1-24}$ (148, 361). Similar substitution in the inactive sequence $ACTH_{1-10}$ induced grooming. In contrast D-enantiomer substitution of Arg in position 8 did not affect grooming behavior (148), pointing to a specific role of the amino acid residue phenylalanine at position 7. The [D-Phe^7] substitution, however, was not effective in all sequences, because the activity of $ACTH_{4-7}$ or $ACTH_{7-10}$ could not be potentiated by [D-Phe] substitution (500).

D-Substitution in peptides is a means to improve their metabolic stability. The fact that the $ACTH_{4-9}$ analogue (Org 2766) that is highly resistant to metabolic degradation and highly active on extinction of avoidance behavior (163, 515) was inactive in inducing grooming behavior (148), however, suggests that improved metabolic stability alone cannot explain the activity of the D-substituted peptides. Interestingly Org 2766 possesses 1,000-fold-less melanotropic activity than $ACTH_{4-10}$ (163). Likewise γ_2-MSH has 1,000-fold-less melanotropic activity than α-MSH (269). Thus excessive grooming may be related to the melanotropic action of ACTH and MSH peptides. This hypothesis is supported by the finding that D-substitution can enhance the grooming-inducing potency (as in [D-Phe^7]$ACTH_{4-10}$): it is known that such substitutions potentiate the melanotropic effects of ACTH peptides (237,

525). An argument against this notion is the fact that the sequence $ACTH_{6-9}$ is essential for melanotropic activity and the structure-activity studies on grooming suggest that the latent activity resides in the sequence $ACTH_{4-7}$.

D. Site of Action in the Brain

The excessive grooming induced by $ACTH_{1-24}$ was reduced in rats bearing lesions in the substantia nigra (140). Large lesions of the hippocampus also reduced grooming, but smaller lesions of either the dorsal or ventral hippocampal area were not effective (81). The lesions reduced the episodes of grooming of long duration but not the grooming episodes per se. No effect on ACTH-induced grooming was found after lesions in the rostral septum, medial septum, or the septal area as a whole; the medial preoptic area; the anterior hypothalamus; the mammillary bodies; the parafascicular nucleus; or the dorsal neocortex. That lesions in the parafascicular area and the dorsal septal area are not effective emphasizes the difference between excessive grooming and avoidance behavior, since these areas are involved in the effect of ACTH fragments on extinction of pole-jumping avoidance behavior (47, 505, 506). Lesions of the amygdala, the mammillary bodies, and the dorsal and ventral hippocampus enhanced ACTH-induced SYS. Lesions of the preoptic region that involved structures for the uptake of [^{3}H]testosterone inhibited ACTH-induced erections and ejaculations but not SYS (27). However, ACTH-induced SYS in guinea pigs was reduced in castrated animals and facilitated by additional testosterone injection [Rodriguez-Sierra et al. (374)].

VI. OTHER BEHAVIORAL EFFECTS OF ACTH-LIKE PEPTIDES

A. Perinatal Influences on Development

Adrenocorticotropin and related peptides have an influence on brain development. Treatment with $ACTH_{1-39}$, $ACTH_{1-24}$, $ACTH_{1-18}$, and $ACTH_{1-16}$ accelerated eye opening, but only when peptides were given on day 3 of life. Smaller peptide fragments were without effect (179). Treatment with α-MSH between days 2 and 7 postnatally significantly improved learning of rats at adult age (384). Increased efficiency in receiving reinforcement with a difficult operant schedule was seen in rats treated postnatally with α-MSH. Rats treated this way acquired and extinguished an active avoidance response faster. A discrimination and reversal problem was also performed more accurately in rats treated in infancy with α-MSH, but the effect was significant in male rats only (22). This had already been observed in a previous study with the potentiated $ACTH_{4-9}$ analogue Org 2766 (76). Female

rats treated postnatally with α-MSH spent more time in contact with one another when placed in pairs in an open field. The same was found for male animals (22). In relatively high doses, $ACTH_{4-10}$ given at days 2 and 7 after birth induced learning deficits in adult life. A decreased performance in active avoidance learning and in reversal of a visual discrimination task was observed (385).

Daily injections of $ACTH_{4-10}$ during the early postnatal period (days 2–8) enhanced the retention of a one-trial passive avoidance response tested in adulthood. Furthermore $ACTH_{4-10}$ attenuated the increment in motility as a result of postnatal manipulation during both active and passive avoidance conditioning (intertrial activity, approach frequency) (325).

B. Other Behavioral Effects

Certain forms of agonistic behavior such as aggressive, submissive, and defensive responses in competitive situations are affected by ACTH (400). Social hierarchy and conflict within this hierarchy influence pituitary-adrenal function (59). This is of interest, because $ACTH_{1-24}$ administered to male mice reduced isolation-induced aggressiveness (61). Although $ACTH_{4-10}$ treatment failed to affect isolation-induced aggression (60), the effect appeared to be extra-adrenal. Leshner et al. (262) found that the effect of ACTH on agonistic behavior is effective in adrenalectomized rats maintained on corticosterone and is independent of the testes. Poole and Brain (348) showed that ACTH causes sham-operated as well as adrenalectomized mice to be subordinate in an isolation-induced aggression paradigm. Administration of α-MSH was reported to lower the aggression level in groups of mice as determined by attack latencies (340).

Adrenocorticotropin and related peptides also affected social behavior in rats. Active social interactions were sniffing, nipping, grooming, mounting, boxing, wrestling, jumping on, and climbing under or over the partner. Sitting or lying together with body contact was regarded as passive contact. Normally a decrease in social interaction occurs when rats are exposed to a brightly lit arena, and anxiolytic drugs prevent or reduce this decrease in social interaction. However, $ACTH_{1-24}$ had the opposite effect: this peptide reduced social interactions (116). Because no decrease in spontaneous locomotor activity was found, it was suggested that ACTH might have an anxiogenic effect (117). The peptide $ACTH_{4-10}$ reduced active social interactions in familiar and unfamiliar environments at low-light and high-light conditions. [D-Phe^7]$ACTH_{4-10}$ also reduced the overall level of active social interaction but only in the unfamiliar situation. This peptide lowered the level of locomotor activity in the test situation and reduced the change in behavior seen with manipulation of the environment. No effects on passive social contact were observed, which suggested a lack of sedative effect of the peptides. It was concluded that the effect of $ACTH_{4-10}$ on social behavior is not secondary to glucocorticosteroid secretion.

In studies on the antagonism of pentobarbital anesthesia by ACTH-like peptides, it was found that $ACTH_{4-10}$ and $ACTH_{4-7}$ were inactive after both peripheral and central injection (32). Both the $ACTH_{4-7}$ amide and the behaviorally potent $ACTH_{4-9}$ analogue Org 2766 were active regardless of the route of administration. These peptides shortened barbiturate-induced sleeping time and hypothermia, showing significant analeptic activity of the same order of magnitude as that of thyrotropin-releasing hormone.

Spontaneous behavior of rats in a novel environment seems not to be influenced by ACTH and its fragments (45, 315, 477, 517), although ACTH and $ACTH_{4-10}$ have been observed to reduce exploration in a holeboard (115). Kastin et al. (223) found that α-MSH failed to influence locomotor activity. Nockton et al. (322), however, found a tendency toward increased activity in an open-field situation in rats receiving α-MSH and footshock prior to the test. The α-MSH appeared to increase locomotor activity of rats with lesions in the septal area (64). Increased locomotor activity was also found by Sakamoto (380) in mice receiving β-MSH; synthetic α-MSH and ACTH appeared to be ineffective. In the rat β-MSH induced drowsiness, hyperpnea, hypertension, and piloerection (380), whereas in the rabbit drowsiness, hyperpnea, and hypotension have been observed after β-MSH or ACTH (102). Amir et al. (7) found that high doses of $ACTH_{1-24}$ (100 or 200 μg sc) depressed and lower doses (30 μg/kg sc) stimulated motor activity in an open field. Naltrexone inhibited the suppressing effect of ACTH, which suggests that opiate receptors are involved in the depressing influence of ACTH on locomotor activity. These observations indicate that the effect of ACTH and related peptides on locomotor activity depends on both the structure of the peptide and the species studied. These effects, however, are elicited by doses of ACTH and related peptides much higher than those needed to affect conditioned behavior (483).

Locomotion, jumping, squeaking, and irritability in mice induced by L-dopa were potentiated by α-MSH. Tremors, head movement, abducted limbs, and irritability induced by 5-HT plus pargyline were not affected by α-MSH. Tremors caused by oxotremorine were also not influenced by α-MSH. However, the peptide slightly increased footshock-induced fighting and decreased audiogenic seizures in susceptible mice (346). Duration of tonic immobility in lizards was decreased by α-MSH and $ACTH_{4-10}$. No significant changes in body temperature resulted from the administration of these peptides, and skin darkening produced by α-MSH had no indirect effect on tonic immobility. Both α-MSH and $ACTH_{4-10}$ were considered to affect fear reduction (425).

VII. EFFECTS OF ENDORPHINS AND RELATED PEPTIDES

A. Active Avoidance Behavior

In view of the potent behavioral effects of ACTH-like peptides, a program was started around 1970 to isolate neuropeptides with behavioral ac-

tivity from hog pituitary material (256). The biological activity of the fractions isolated was assayed on extinction of active and passive avoidance behavior. One of the peptides obtained in pure form yielded three small peptides on tryptic digestion. The amino acid composition of two of these fragments, as was later realized, had a striking similarity to that of β-LPH_{61-69} and β-LPH_{70-79}. One of the peptides, with the amino acid composition of β-LPH_{61-69}, possessed potent activity in the behavioral tests, but the amount available at the time was small and did not allow structural analysis. Several years later it was discovered that peptides with high affinity for opiate-binding sites and other opiatelike characteristics occur naturally in pituitary and brain (56, 167, 193). These peptides, designated as enkephalins and endorphins, appeared to be structurally related to COOH-terminal sequences of β-LPH.

Extinction of pole-jumping avoidance behavior was used to assay the behavioral effect of the endorphins. It was found that these peptides, like ACTH and neurohypophyseal hormones, profoundly affect active and passive avoidance behavior. After subcutaneous injection, Met-enkephalin appeared to be as active as $ACTH_{4-10}$, but α-endorphin (β-LPH_{61-76}) and β-LPH_{61-69} were much more potent. However, $ACTH_{4-10}$ and α-endorphin did not differ in potency on extinction of pole-jumping avoidance behavior when administered intracerebroventricularly (487). It appeared that the influence of similarly administered β-endorphin on pole-jumping avoidance behavior (491) could not be blocked by the specific opiate antagonist naltrexone. This suggested that the influence of endorphins on avoidance behavior is independent of opiate receptors (491). Rigter et al. (371) showed that peripherally injected Leu- and Met-enkephalin and [D-Ala^2-D-Leu^5]enkephalin but not [D-Ala^2]Met-enkephalin impaired acquisition of a one-way active avoidance response. Naloxone blocked this effect of Leu-enkephalin only when very high doses were used.

That endorphins influence CNS processes involved in learning was also shown by Gorelick et al. (155). These authors found that chronic administration of nonanalgesic amounts of β-endorphin did not affect the number of trials to achieve the learning criterion, but the response latencies were significantly longer. When moderate doses of β-endorphin were injected intraperitoneally immediately after training in a shuttle-box avoidance task, a retrograde amnesia was observed (200). When the peptide was given during the training, no effect on acquisition was found; when given before the test session, however, retrieval was facilitated (199). A marked depletion of immunoreactive β-endorphin in extrahypothalamic brain tissue was found after completion of the training in the shuttle box. On the other hand peripheral injection of relatively low amounts of α- or γ-endorphin did not affect acquisition of shuttle-box avoidance behavior (39), although high doses of γ-endorphin have been shown to retard learning in the shuttle box (235).

It has been hypothesized that β-endorphin may contain sequences with opposite behavioral effect, because β-endorphin was less potent than α-endorphin in delaying extinction of pole-jumping avoidance behavior. Indeed

γ-endorphin appeared to have behavioral effects opposite to those of α-endorphin, in that it facilitated extinction of pole-jumping avoidance behavior (493). Removal of the NH_2-terminal tyrosine, which eliminated the opiatelike activity of the endorphins (121), yields β-LPH_{62-77} (DTγE). This peptide had retained the effect on avoidance behavior and appeared to be even more potent than γ-endorphin. Systemic injection of 0.01 μg to rats caused a significant effect on extinction of pole-jumping avoidance behavior. Subsequently the effect of DTαE on extinction was found to be comparable to that of α-endorphin. Thus γ-endorphin and α-endorphin (and DTγE and DTαE) possess opposite effects on extinction of pole-jumping avoidance behavior. LeMoal et al. (258) also found that α- and γ-endorphin respectively inhibited and facilitated extinction of pole-jumping avoidance behavior.

B. Passive Avoidance Behavior

Long-term retention of a passive avoidance response was facilitated by posttrial injection of Met-enkephalin (411). Leu-enkephalin was not active, and the effect of Met-enkephalin was not naloxone reversible. When the peptide was not administered immediately after the trial but after a delay of 15 min, no effect was found. It was therefore concluded that the peptide facilitates consolidation of memory.

Experiments on amnesia also suggested that enkephalins affect memory. Met-enkephalin given systemically in relatively low doses either before acquisition or before retention testing reduced CO_2-induced amnesia for a one-trial passive avoidance response (370). Leu-enkephalin was only active when given before the retention test. These antiamnesic effects of the enkephalins could not be prevented by naloxone. These findings contradict those of Izquierdo et al. (200), who proposed that the endorphins are amnesic agents. These authors found that moderate amounts of Leu-enkephalin or β-endorphin given intraperitoneally after the training induced retrograde amnesia. Low doses of β-endorphin injected immediately after the learning trial also impaired the retention. No such effect was found when the treatment was postponed for 90 min after the learning trial. Leu-enkephalin and the D-Ala-D-Leu analogue facilitated acquisition of an inhibitory avoidance response but had no effects on acquisition of a swim escape response (372). Enkephalins may suppress behavior in the presence of cues that were previously associated with aversive stimuli.

Kovács and de Wied (245) found that β-endorphin has a complicated effect on passive avoidance behavior. In a relatively small dose (1.5 μg sc) given immediately after the learning trial, the peptide slightly facilitated passive avoidance behavior as measured by the retention test 24 h later. A dose of 10 μg was much more active. When β-endorphin was injected 1 h before the retention test, however, 1.5 μg caused a significant effect, but the 10-μg dose was inactive. The opiate antagonist naltrexone slightly attenuated

passive avoidance behavior. It did not prevent the facilitation of passive avoidance behavior induced by β-endorphin, suggesting that the effect of this peptide on conditioned behavior does not involve opiate receptors.

Apparently α-endorphin facilitated but γ-endorphin or DTγE attenuated passive avoidance behavior. Such opposite effects had also been found in the pole-jumping active avoidance behavior paradigm. The exact moment of peptide administration seemed important for the behavioral effect, since γ-endorphin enhanced retention of a one-trial inhibitory avoidance task when injected intraperitoneally 30 min prior to the training but not when given immediately after or 90 min before training (285). This finding is not in accordance with results found by Kovács and de Wied (245), who studied the time-gradient effect of α- or γ-endorphin using the one-trial passive avoidance test. Both peptides were highly active when given immediately after the learning trial. When given after 3 h a slight effect was found, and they were inactive when administered 6 h after the learning trial. When given 1 h prior to the retention trial both peptides were highly active again, α-endorphin in facilitating and γ-endorphin in attenuating passive avoidance behavior (245). Because consolidation of newly acquired information is generally believed to take place within the first 3 h after learning (290), this suggests in the first place that α- and γ-endorphin influence memory consolidation. In addition an influence on memory-retrieval mechanisms is probable, in view of the effects observed when the peptides were injected 1 h prior to the retention test. A similar effect on retrieval has been found with the neurohypophyseal hormones vasopressin and oxytocin (489). However, differences between the influence of the endorphins and the neurohypophyseal hormones on problem-solving behavior (40, 245) suggest that the latter hormones are more specifically involved in memory processes than the endorphins.

C. Positively Reinforced Behavior

γ-Endorphin significantly facilitated problem-solving behavior in a food-rewarded multiple-maze situation (40), because animals treated with this peptide made fewer errors than saline-treated controls. α-Endorphin-treated rats, however, made more errors than saline-treated controls. This suggests that these two fragments influence cognitive processes in an opposite way (40).

In a lever task involving food reward, α-endorphin delayed but γ-endorphin facilitated extinction (238). In a runway task involving water reward, however, both α- and γ-endorphin delayed extinction. The effect of the two peptides was not blocked by naloxone (258). On the other hand DTγE facilitated extinction in a food-reinforced runway task, whereas α-endorphin and DTαE were inactive (165).

Effects of experiments with Met-enkephalin analogues demonstrated that the opiatelike effects of the endorphins are dissociated from the be-

havioral effects. This was first demonstrated by Kastin et al. (225), who found that hungry rats treated with Met-enkephalin, [D-Ala^2]Met-enkephalin, and the nonopiate [D-Phe^4]Met-enkephalin for 3 days negotiated a complex 12-choice Warden maze significantly faster and with less errors than placebo-treated rats. This conclusion was confirmed in later studies (370, 491). Centrally administered β-endorphin suppressed fixed-ratio operant conditioning for food reinforcement (268). Furthermore subcutaneously administered [D-Ala^2, F_5-Phe^4]Met-enkephalinamide facilitated learning of a discrimination-reversal task for a food reward in rhesus monkeys. This Met-enkephalin analogue did not influence general activity, short-term memory, startle response, or analgesia (333). These effects on discrimination learning were interpreted as caused by enhanced cognitive flexibility rather than improvement of short-term memory or associative information.

Self-administration of Met-enkephalin into the lateral ventricle of the rat has been reported by Belluzzi and Stein (24). These authors suggested that the enkephalins influence reward mechanisms. Both the acquisition and the maintenance of self-administration behavior was achieved after the intraventricular administration of Leu- and Met-enkephalin, but [des-Tyr^1]-Met-enkephalin was ineffective (24). In addition facilitation of electrical self-stimulation was found, particularly in brain regions rich in enkephalin immunoreactivity. The self-stimulation in these areas was counteracted by naloxone but also by pretreatment with the norepinephrine-synthesis inhibitor diethyldithiocarbamate, suggesting that norepinephrine is involved as well. Intravenous self-administration of the synthetic enkephalin analogue FK-33-824 was found in morphine-dependent rhesus monkeys as well (295). Van Ree et al. (357) found the same for β-endorphin but not for Met-enkephalin in rats. Interestingly, Van Ree et al. (357) also found that injection of β-endorphin into the nucleus raphe magnus acted as a discriminative stimulus in rats trained to discriminate fentanyl from saline (357).

[D-Ala^2]Met-enkephalinamide administered either intravenously or intracerebroventricularly in morphine-dependent rats maintained self-administration on a level like that after morphine treatment. No signs of abstinence were observed during enkephalin substitution, so it was suggested that [D-Ala^2]Met-enkephalinamide serves as a reinforcer of opiate-seeking behavior and maintains physical dependence in rats (446). This enkephalin analogue was also used to test the relationship between the action of enkephalin and the reinforcing action of electrical stimulation in the posterior lateral hypothalamus (330). Rats were implanted with a combination of a cannula and a stimulation electrode. [D-Ala^2]Met-enkephalinamide was readily self-administered in those rats showing self-stimulation at moderate to high rates. The higher the dose of the peptide, the stronger was the rate of self-administration, and naloxone (but not naltrexone) blocked this reinforcing effect of the [D-Ala^2] analogue.

Finally, DTγE attenuated electrical self-stimulation elicited from the ventral tegmental area or the nucleus accumbens (95, 356) and reduced ac-

quisition of heroin self-administration (355). Conversely α-endorphin facilitated electrical self-stimulation from the ventral tegmental area (95) and slightly facilitated acquisition of heroin self-stimulation (355).

D. Sexual Behavior

Subcataleptic doses of β-endorphin administered into a lateral cerebral ventricle appeared to affect copulatory behavior of the male rat (299). Treated animals were less likely to initiate mounting, and intromissions rarely occurred. The opiate antagonist naltrexone appeared to block this effect of the peptide, indicating that opiate receptors are involved. Likewise, [D-Ala^2]Met-enkephalin administered intracerebroventricularly in microgram amounts inhibited copulatory behavior of sexually vigorous male rats (135). Naloxone prevented the effect of this enkephalin analogue. It was therefore suggested that endogenous opiatelike peptides may be responsible for sexual inhibition in the rat. Naloxone was able to induce copulatory activity in sexually inactive male rats but failed to influence the behavior of sexually active males. (135). On the other hand a facilitation of copulatory activity was observed after peripheral administration of 15 μg of [D-Ala^2]Met-enkephalinamide (38). The predominant effect was on ejaculation latency. The same dose of Met-enkephalin failed to influence copulatory activity, presumably because of its rapid enzymatic degradation.

VIII. OTHER BEHAVIORAL EFFECTS OF ENDORPHINLIKE PEPTIDES

A. Opiatelike Effects

Opiates produce a variety of behavioral effects ranging from behavioral activation to immobility. All these effects are found with the endorphins as well. Bloom et al. (34) studied the effect of Met-enkephalin and α-, β-, and γ-endorphin after ICV injection and found that β-endorphin produced a markedly prolonged muscular rigidity and immobility similar to a catatonic state. All peptides evoked wet-dog shaking in a dose-related manner. This effect was reversible by naloxone. Interestingly, whereas β-endorphin produced hypothermia in the doses used, γ-endorphin induced hyperthermia. The authors suggested that alterations in the homeostatic regulation of β-endorphin could have etiological consequences in mental illness. Jacquet and Marks (205) came to similar conclusions. These authors injected β-endorphin in the periaqueductal gray of the rat and found profound sedation and catalepsy. Microinjection of smaller endorphin fragments caused attenuated forms of this behavior. The similarity between β-endorphin effects and those of neuroleptics led these authors to suggest that a disturbance in the bioavailability of this neuropeptide to receptor sites in the brain might be an

etiological factor in those psychopathological states for which exogenous neuroleptics exert an ameliorative influence.

The behavioral effects of all opiatelike peptides resemble those of morphine, provided that sufficiently high amounts of the peptide are used or peptides protected against enzymatic degradation: extremely high doses of Met-enkephalin caused immobility that could be prevented by naloxone (77). Furthermore [D-Met^2-Pro^5]enkephalin, which is a potent analgesic, induced catalepsy, exophthalmus, deep sedation, piloerection, and hypothermia (430). Similar effects are found with [D-Ala^2, F_5-Phe^4]Met-enkephalinamide (333). Intracerebroventricularly injected β-endorphin or [D-Ala^2-D-Leu^5]enkephalin induced loss of the righting reflex in the golden hamster (448) and catalepsy in the rat. When injected into the cisterna magna, the periaqueductal gray, or the lateral ventricle, β-endorphin caused catatonia or catalepsy in addition to analgesia. These effects could be blocked by naloxone and also by L-dopa combined with a peripheral decarboxylase inhibitor or apomorphine. The latter two treatments did not prevent analgesia, suggesting that part of the effect of β-endorphin occurs by blocking dopaminergic neurons (202). A good correlation was found between the anatomy of the endogenous β-endorphin system and the brain sites in which microinjection of β-endorphin caused catalepsy. Injections into the enkephalin system did not result in analgesia, catalepsy, or changes in body temperature (449). Injection of β-endorphin into the substantia nigra produced constant sniffing, licking, and biting behavior similar to the effects of apomorphine. This was not observed after injection into the basal ganglia or the nucleus accumbens (449). β-Endorphin infused into the ventral tegmental area stimulated the locomotor activity of the rat (411). Both α- and γ-endorphin and also DTγE caused a similar effect, whereas the effect of the [D-Ala^2] analogue of α- and γ-endorphin was stronger and longer lasting. This locomotor activity was blocked by either pretreatment with naloxone or by destruction of the terminal projections of the mesolimbic dopamine (DA) system that originate in the ventral tegmental area. Interestingly in this study α- and γ-endorphin caused similar effects, whereas the effect of DTγE was also naloxone reversible. This suggests that effects on locomotor activity may be located in smaller fragments of the endorphin molecule common to all four peptides used in these experiments and that the effect is mediated in some way by DA, which affects opioid activity.

Endorphins, when used in lower doses, have an activating influence on behavior (34, 402). For example, Leu-enkephalin induced a naloxone-insensitive rotational behavior (77). Intracerebroventricularly injected [D-Ala^2]Leu- or Met-enkephalin increased locomotor activity in mice, and naloxone blocked this behavior. Naloxone also antagonized the novelty-induced increase in locomotor activity (229). Wet-dog shaking and scratching were reported in response to subcataleptic ICV doses of β-endorphin in rats (268, 476). In addition copious salivation and a seizurelike state were induced by the peptide (190). The mesolimbic DA system appears to be involved in this

behavioral activation, since β-endorphin injected in the vicinity of the cell bodies of the dopaminergic cells in the ventral tegmental area induced a behavioral activation similar to that produced by systemically administered opioids (63, 231, 413). Increased sniffing and grooming, interrupted by bursts of locomotor activity, were found (222) and in addition rearing and walking were increased. These effects were naloxone reversible. Neuroleptic drugs or 6-hydroxydopamine (6-OHDA) lesions of the mesolimbic dopamine system blocked these effects (231, 413).

Substantial behavioral effects of endorphins and related peptides have been found after systemic administration. In cats, β-endorphin produced a dose-dependent inhibition of the jaw-opening reflex. The response to nociceptive stimuli applied to the skin was also inhibited by the peptide. Fine tremors, mild excitation, visual hallucinations, fixation of the eyes in space or on an object, and failure to respond to auditory stimuli were observed (293). A decrease in locomotor activity was found in squirrel monkeys after intracisternal and intravenous injection of [D-Ala^2]Met-enkephalin. The effect was more pronounced and less variable after central administration. Other effects noted were decreased aggression after central administration and increased distress vocalization after intravenous injection. Increased responsiveness to air puffs and light after central application of β-endorphin was also found. The time needed to pick up an apple was not substantially affected by either treatment (333). High-frequency licking and reduced locomotion were observed in cats after intravenous administration. At high doses (100–500 μg/kg) the responses included vomiting and relaxation of the nictitating membrane. These effects appeared to be mediated by opiate receptors, since they were naloxone reversible (75). The open-field behavior of male rats was affected by intraperitoneal injection of 100 μg of α-, β-, or γ-endorphin or their [D-Ala^2] analogues. β-Endorphin increased grooming and the [D-Ala^2] analogue enhanced sexual arousal. Rats injected with γ-endorphin or with the [D-Ala^2] analogue ran to the wall of the open field faster and produced more fecal boli, suggesting heightened emotionality (457). These effects were naloxone reversible. β-Endorphin was found to depress activity of rats in their home cage, whereas in novel situations, activity seemed increased by the peptide (234). In another study β-endorphin, DTγE, and α-endorphin did not substantially affect behavior in an open field after subcutaneous injection in doses five times those needed to produce effects on active and passive avoidance behavior (493). However, the responsiveness of rats to electric footshock was changed by the peptides (297). Graded doses of β-, γ-, and α-endorphin and fragments of these neuropeptides were subcutaneously administered. γ-Endorphin, DTγE, DTαE, β-LPH$_{65-77}$, β-LPH$_{62-69}$, and [D-Phe^7]ACTH$_{4-10}$ reduced the number of occurrences in which the animal did not respond to the shock. Met-enkephalin, α-endorphin, β-endorphin, β-LPH$_{70-77}$, and ACTH$_{4-10}$ were not active, although a dose of β-endorphin 10 times higher appeared to increase the occurrence of no response. This effect was also found with morphine and may be related to the

antinociceptive action of these compounds. It was concluded that endorphins related to γ-endorphin increase the sensitivity to electric footshock. This effect is independent of opiate receptors. Furthermore structure-activity studies revealed that the sequence β-LPH_{65-69} may contain the active core for this activity, although fragments containing the amino acid residue leucine in position 77 were more active than peptides lacking this amino acid.

B. Neurolepticlike and Amphetaminelike Activity

Jacquet and Marks (205) suggested that β-endorphin might be a neurolepticlike peptide and that a disturbance in the bioavailability of this peptide in the brain may be an etiological factor in psychopathology. This hypothesis emerged from experiments in which β-endorphin was injected directly into the periaqueductal gray. These findings, however, were challenged by Segal et al. (402). These authors compared the effects of β-endorphin and the neuroleptic drug haloperidol. The peptide was injected intraventricularly or directly into the periaqueductal gray, and it was found that β-endorphin caused a period of rigid immobility that was preceded and followed by hyperactivity. The immobility was accompanied by loss of righting reflex. In contrast to β-endorphin, no dose of the neuroleptic drug haloperidol produced rigidity, loss of righting reflex, or behavioral excitation. Rats treated with β-endorphin typically slid off a vertical grid; haloperidol treatment facilitated their grip on the grid, and the β-endorphin–induced rigid immobility was counteracted by haloperidol when the two compounds were given simultaneously. These results were in essence confirmed by de Wied et al. (493), who compared the effects of β-endorphin with morphine and haloperidol. Spontaneous behavior, the appearance of the eyes, the state of reflexes, and the activity in the so-called bridge test (34) and in various grip tests revealed that β-endorphin resembled morphine rather than haloperidol.

Neurolepticlike activity was later found for DTγE; in contrast DTαE resembles amphetamine in its behavioral effects (355, 493). Thus DTγE facilitated extinction of pole-jumping avoidance behavior and attenuated passive avoidance behavior, as did haloperidol (244). This peptide also induced a positive grasping response, and thus treated rats displayed a slight immobility in their home cage. However, the reduction in locomotor activity and the sedation seen with haloperidol were not induced by DTγE. Dorsa et al. (95) and van Ree and Otte (356) showed that DTγE attenuated electrical self-stimulation elicited from the ventral tegmental area or the nucleus accumbens. Haloperidol had the same effect. However, the peptide affected electrical self-stimulation at threshold current only, whereas haloperidol reduced self-stimulation rates both at threshold current and at currents eliciting maximal performance. Moreover both haloperidol and DTγE (but not α-endorphin) inhibited ACTH-induced excessive grooming behavior of rats when injected into the neostriatum or the nucleus accumbens (85, 140).

Furthermore both DTγE and haloperidol decreased acquisition of heroin self-administration (355). Although these findings are in line with the notion that DTγE has neuroleptic activity, the issue is not settled, in view of negative findings reported by Weinberger et al. (478). These authors failed to find evidence for an effect of DTγE comparable to that of haloperidol. Whereas haloperidol pretreatment decreased amphetamine-induced locomotor activity, DTγE even in high doses did not. The same held for apomorphine. No catalepsy was found with DTγE, and this peptide reduced only the number of rearings in a multicompartment chamber in which haloperidol reduced locomotion. Furthermore haloperidol stimulated the conversion of tyrosine to dopamine in the caudate nucleus, but DTγE had no effect. Finally, DTγE did not appear to be positive on a vertical grid (478). The notion that DTγE might have neuroleptic-like activity suggested that the peptide might also have antipsychotic effects. This hypothesis was investigated in three clinical studies involving 23 patients suffering from chronic relapsing schizophrenic or schizoaffective psychosis (358, 460). These studies substantiated the notion that DTγE might possess antipsychotic activity.

The peptide DTγE cannot be regarded as a classic neuroleptic drug because it does not displace [^{3}H]spiperone or [^{3}H]haloperidol or [^{3}H]apomorphine from their specific binding sites in brain membrane preparations (359). Pedigo et al. (341) also failed to find effects on the binding of [^{3}H]spiroperidol in the corpus striatum, frontal cortex, nucleus accumbens, and olfactory tubercle of the rat. However, a decrease in [^{3}H]spiperone binding in vivo has been found after systemic injection of high amounts of DTγE. The corpus striatum and nucleus accumbens were particularly affected, and the authors suggested the DTγE may release endogenous dopamine or alter the conformation of neuroleptic binding sites. Alternatively DTγE may exert its effect through an active metabolite. Interference with dopaminergic systems in the brain by DTγE was also described by Nickolson et al. (318). Apparently DTγE depressed the K^+-evoked DA release from brain slices in vitro; DTγE also potentiated apomorphine-induced yawning (318). On the other hand haloperidol increased both basal and K^+-evoked DA release (393). The suggestion that β-LPH_{66-77} ([des-enkephalin]γ-endorphin, DEγE) is the principal metabolite of DTγE is interesting in view of recent structure-activity studies. Apparently DEγE is the shortest peptide sequence that is active on pole-jumping avoidance behavior and is as potent as DTγE in the grip tests (494). Thus not only the NH_2-terminal tyrosine but the whole enkephalin molecule can apparently be removed without loss of the behavioral activity of the peptide, since enkephalin has an effect on extinction opposite to that of the γ-type endorphins (491). It is possible that DEγE is an endogenous neurolepticlike neuropeptide. It is well known that neuroleptic drugs are antidopaminergic substances. In addition the antipsychotic action of neuroleptics may be related to their dopamine-blocking activity. It is therefore important that DEγE dose-dependently antagonized the reduction in ambulation rate induced by low doses of apomorphine (360). This suggests

that DEγE interferes with presynaptic dopaminergic receptors. It was also found that DEγE possesses antipsychotic effects in schizophrenic patients (358), and it was suggested that DEγE rather than DTγE might play a key role in psychopathology (494).

The behavioral effects of the α-type endorphins are opposite to those of the γ-type endorphins and in some respects resemble those of the psychostimulant drug amphetamine (355). Thus α-endorphin delayed extinction of pole-jumping avoidance behavior and facilitated passive avoidance behavior (493), as did amphetamine (244). In addition α-endorphin facilitated electrical self-stimulation elicited from the ventral tegmental area (95) at threshold currents only, whereas amphetamine facilitated performance both at threshold and maximal current intensities. The peptide, however, did not mimic the effects of amphetamine on locomotor behavior and stereotypy (244, 355, 478, 493). Furthermore α-endorphin was not active on electrical self-stimulation elicited from the nucleus accumbens, in contrast to amphetamine, which enhanced electrical self-stimulation elicited from the accumbens area (356). α-Endorphin also slightly facilitated acquisition of heroin self-administration (355). Structure-activity studies showed that the removal of the NH_2-terminal amino acid residue tyrosine of α-endorphin did not modify the influence of this peptide on extinction of pole-jumping avoidance behavior (164). Removal of the whole enkephalin moiety, however, markedly affected the activity of α-endorphin on extinction of pole-jumping avoidance behavior, since β-LPH_{66-76} was much less active in this respect.

C. Endorphins and Excessive Grooming

The peripheral injection of low doses of morphine into the rat is followed by increased grooming and scratching (16). Gispen and Wiegant (147) showed that morphine also induced grooming when injected intracerebroventricularly in relatively low doses. This effect was done in hypophysectomized rats as well as in intact rats, and the same observations were done for β-endorphin. This peptide was more potent than $ACTH_{1-24}$ in producing excessive grooming (149): the opioid induced the grooming response in doses as low as 10 ng. The nature of the excessive grooming induced by β-endorphin appeared to be somewhat different from that induced by ACTH: the β-endorphin grooming is frequently interrupted by signs of excitation (quick movements of body and head, jumping and gnawing, and body shakes). In addition ACTH increased the duration of grooming bouts but not the number of grooming bouts per observation period, whereas β-endorphin did not change the duration of grooming bouts, but it increased the number of bouts per observation period (140). A further difference from ACTH-induced grooming is that no SYS was observed after β-endorphin.

Structure-activity studies were performed with fragments of β-endorphin. Shortening the β-endorphin from the COOH-terminal end resulted in

a progressive loss of activity. Both α-endorphin and γ-endorphin possessed slight grooming-inducing potency, whereas β-LPH_{61-69} was the shortest sequence with some activity. Met-enkephalin was inactive, even if injected in high doses (149). The [des-Tyr] fragments, that lack opiatelike activity (121, 167) were also inactive, again underscoring the opiatelike character of peptide-induced grooming. Similarly peripheral administration of specific opiate antagonists (naloxone, naltrexone) completely inhibited the grooming induced by $ACTH_{1-24}$ (147) and by β-endorphin (149). Likewise the acute tolerance that develops after β-endorphin was also reversed by naloxone (496).

D. Perinatal Effects on Development

Injection of β-endorphin into rats from day 2 through day 7 after birth appeared to increase the threshold for heat but not for shock-induced pain when these animals were tested at 90 days of age (388). Rats treated with relatively high doses of Met-enkephalin (given directly after birth) negotiated a maze significantly better than saline-treated controls (385). In addition when pregnant females were treated with β-endorphin every other day from day 7 through day 21 of pregnancy, their offspring showed a retarded development (292). Eye opening was significantly delayed, and these rats were less active and less responsive to environmental stimuli. The startle response to a very loud stimulus was markedly attenuated, and behavior in an open field was also different in these rats, with reduced exploration and less time spent in physical contact. Furthermore passive avoidance behavior was facilitated 24 h after a single shock exposure, but the rats required significantly more trials to reverse on an initial learning task.

IX. BEHAVIORAL EFFECTS OF γ_2-MSH

The NH_2-terminal part of the pro-opiocortin molecule contains some peptide sequences with a primary structure resembling that of α- and β-MSH (317). These sequences are located between pairs of basic amino acids in the cryptic region of the pro-opiocortin molecule. A sequence of 27 amino acids has been termed γ-MSH. The amide of this amino acid sequence has been designated as γ_1-MSH. The hydroxylated sequence Tyr-Val-Met-Gly-His-Phe-Arg-Trp-Asp-Arg-Phe-Gly-OH was termed γ_2-MSH (269). These peptides were synthesized and studied in various tests. The terminology used may be incorrect, because the melanotropic activity of γ-MSH is low compared with α-MSH (269). In four of the tests γ_2-MSH affected behavior in a way opposite to α-MSH. It facilitated extinction of pole-jumping avoidance behavior and attenuated passive avoidance behavior when injected before the retention test. It also decreased the rate of acquisition of shuttle-box avoidance behavior. Furthermore, in contrast to α- and β-MSH, γ_2-MSH

failed to induce excessive grooming after intraventricular administration (353). However, it was able to attenuate $ACTH_{1-24}$-induced excessive grooming after subcutaneous administration. The same study showed that γ_2-MSH elicited symptoms resembling opiate withdrawal when injected into the periaqueductal gray and appeared to be much more potent than $ACTH_{1-24}$ (203). Wet-dog shaking, teeth chattering, eye twitching, and increased ambulation and grooming were found. In addition γ_2-MSH caused symptoms that were not seen after $ACTH_{1-24}$, such as increased sniffing, licking of the penis, and scream on touch. These effects are qualitatively and quantitatively different from those seen in morphine-dependent rats treated with naloxone.

Further studies revealed that γ_2-MSH attenuated some effects of intracranially administered β-endorphin. In particular the decrease in spontaneous behavior and also the analgesia and changes in core temperature induced by β-endorphin were attenuated after ICV injection of γ_2-MSH (353). In addition the β-endorphin–induced rise in circulating α-MSH (294) was prevented by γ_2-MSH. Effects on analgesia, core temperature, and release of α-MSH were also blocked by naloxone. Injections of γ_2-MSH also suppressed heroin self-administration (353).

Studies on the interaction between γ_2-MSH and β-endorphin in vitro revealed that γ_2-MSH in micromolar amounts was able to displace radioactive naloxone from its specific binding sites in brain membrane preparations. In this respect it is a weaker antagonist than $ACTH_{1-24}$ (329). However, γ_2-MSH did not counteract the effect of β-endorphin on the stimulated guinea pig ileum (relaxation) and on the rat rectum (contraction). This latter tissue appeared to be very sensitive to γ_2-MSH: the peptide itself induced relaxation (353). From these experiments it was concluded that γ_2-MSH at least in some aspects may operate as a functional antagonist of β-endorphin. A similar conclusion has been drawn about $ACTH_{1-24}$ and related peptides (143, 385, 437).

X. BIOTRANSFORMATION OF NEUROPEPTIDES DERIVED FROM PRO-OPIOCORTIN

It has been proposed that pro-opiocortin is not only a precursor molecule for ACTH, MSH, β-LPH, and β-endorphin but also for other behaviorally active neuropeptides. Because the amino acid sequence of part of β-LPH (residues 41–58) resembles that of β-MSH, Li et al. (267) suggested that β-LPH is a possible prohormone for β-MSH. Other fragments of β-LPH such as γ-LPH (β-LPH_{1-58}), N-fragment (β-LPH_{1-38}), C′-fragment (β-LPH_{61-87}), and C-fragment (β-LPH_{61-91}) were subsequently found in bovine pituitary glands as intact peptides (53, 55). Each of these peptides seems to be formed by cleavage of the lipotropin chain at the carboxyl side of paired basic amino acid residues (Lys-Lys and Lys-Arg) and subsequent removal of the basic amino acids. Thus pituitary enzymes resemble other enzymes that generate hormones from their prohormones [e.g., insulin from proinsulin, (232)]. The

presence of several basic pairs in the primary structure of pro-opiocortin as proposed by Nakanishi et al. (317) suggests that this protein can be attacked by such proteolytic enzymes. Some families of shorter peptides are thus generated. Adrenocorticotropin, β-LPH, and γ-MSH are formed: ACTH may subsequently be cleaved to yield α-MSH via intermediates such as $ACTH_{1-16}$ and CLIP (401); β-LPH is cleaved into γ-LPH and β-MSH on the one hand and β-endorphin on the other; β-endorphin again may be the precursor of α- and γ-endorphin and related peptides (65).

Not much is known about the generation of ACTH fragments such as $ACTH_{4-10}$ that appear to contain the intrinsic behavioral activity of the ACTH molecule. Among the behaviorally active fragments of ACTH are $ACTH_{4-7}$, $ACTH_{4-10}$ (β-LPH_{47-53}), $ACTH_{1-10}$, α-MSH (*N*-acetyl-$ACTH_{1-13}$ amide), and $ACTH_{1-16}$. The fragment $ACTH_{1-39}$ is present in the anterior pituitary, whereas α-MSH and CLIP have been isolated from the intermediate lobe of the pituitary (279, 280, 399, 401). These latter two peptides can be generated in vivo from $ACTH_{1-39}$ via peptidase activity acting in the sequence 15–18, yielding peptides such as $ACTH_{1-16}$ and $ACTH_{17-39}$ (279, 401). It has been proposed that $ACTH_{1-16}$ is further converted to α-MSH by carboxypeptidase action, followed by acetylation and amidation (401). Smaller behaviorally active fragments such as $ACTH_{1-10}$ and $ACTH_{4-10}$ have not yet been reported to be present in the brain or to be generated in vitro.

Not much is known about the formation of ACTH fragments in the body, although various metabolites of exogenously administered $ACTH_{1-39}$ have been found. Aminopeptidase activity caused the removal of the NH_2-terminal amino acid residues Ser-Tyr-Ser from $ACTH_{1-39}$ in muscle and skin. The fragment $ACTH_{3-39}$ was the only metabolite in plasma (192). The same occurred after $ACTH_{1-24}$ administration, in addition to cleavage between the 6–7, 7–8, and 8–9 bonds. A large range of metabolites was formed after cleavage in the COOH-terminal region between the 15–16, 16–17, 17–18, 18–19, 20–21, and 21–22 bonds (191, 192).

The brain is highly active in the proteolytic processing of ACTH. Soluble mouse brain preparations preferentially liberated amino acids from the NH_2-terminal region of $ACTH_{1-24}$ (364). Although the transient formation of larger intermediates could not be ruled out, the pattern of breakdown made their accumulation in vivo unlikely. The presence of enzyme systems that selectively cleave ACTH has yet to be demonstrated. This has already been found for β-endorphin and oxytocin (65, 67).

As mentioned above, the intermediate lobe of the pituitary may form $ACTH_{1-16}$ from $ACTH_{1-39}$ and further convert this peptide to α-MSH. The peptide $ACTH_{1-16}$ is an important fragment of the ACTH molecule with respect to behavioral activity. Analogues of this fragment have been prepared that are a millionfold more potent than $ACTH_{4-10}$ on pole-jumping active avoidance behavior (163). In addition $ACTH_{1-16}$ is as active as $ACTH_{1-24}$ and $ACTH_{1-39}$ in inducing excessive grooming, but $ACTH_{4-10}$ is inactive in this respect. The same is true for a number of neurochemical parameters in which

$ACTH_{1-16}$ appeared to be as active as $ACTH_{1-24}$: modulation of brain adenylate cyclase activity (498, 499); inhibition of protein phosphorylation in a synaptosomal plasma membrane preparation (534); modulation of polyphosphoinositide metabolism in brain membranes (214).

It has been hypothesized (472) that the brain processes the pro-opiocortin molecule in the same way as the intermediate lobe of the pituitary. Indeed if that is the case, $ACTH_{1-16}$ may be the behaviorally active moiety derived from $ACTH_{1-39}$, since $ACTH_{1-39}$ as such is not released from the lobe but α-MSH and CLIP are (279, 399, 401). Whether a peptide such as $ACTH_{1-16}$ is a precursor of smaller fragments ($ACTH_{4-10}$, $ACTH_{4-7}$, $ACTH_{7-16}$) is not yet clear but may be revealed by studies on the biotransformation of this peptide in brain tissue.

The generation of behaviorally active endorphin fragments in the brain has been more extensively documented. The peptide β-LPH_{61-87} (C'-fragment) has been isolated from pituitary tissue (54), and acetylated forms of β-endorphin and β-LPH_{61-87} have also been identified in this gland (406, 407). These acetylated peptides do not possess affinity for brain opiate receptors nor do they produce analgesia.

β-Endorphin-immunoassayable material is secreted into the circulation by the pituitary gland (168). Functional conversion apparently does not occur in the circulation, because β-endorphin was not significantly converted by endopeptidase activity in serum. Furthermore ^{125}I-labeled β-endorphin was resistant to aminopeptidase activity. The shorter fragments, however, were increasingly susceptible (65).

Biosynthesis of the ACTH/β-endorphin precursor has been reported to occur in bovine hypothalamus (270). In addition β-endorphin-immunoreactive cell bodies have been identified in the arcuate nucleus of the hypothalamus (270, 474, 475, 529). β-Endorphin was also found in cerebrospinal fluid (CSF). Aminopeptidase and endopeptidase activity that acts on β-endorphin and Met-enkephalin were not found in the CSF (66), suggesting that these peptides are not subject to proteolytic conversion in CSF. This may indicate that the levels of β-endorphin and related fragments in CSF may reflect changes in metabolism in and release from brain cells. Proteolysis may be abnormal in psychopathology (101).

In synaptosomal plasma membrane (SPM) preparations of rat brain, high endopeptidase as well as aminopeptidase activities have been detected (65), and both enzyme activities were required for the formation of β-endorphin fragments. Austen and Smyth (14), using β-endorphin with the NH_2-terminal tyrosine residue labeled with ^{125}I, have demonstrated that striatal slices and brain membranes convert β-endorphin to γ-endorphin and α-endorphin (15). The experiments were performed in the presence of bacitracin to protect the peptide against release of the labeled tyrosine residue. More recent experiments employed a method that combines the high resolving power of high-pressure liquid chromatography with the extreme sensitivity of radioimmunoassay systems (274) to measure β-endorphin fragments in

brain and CSF (458). It was demonstrated that α-endorphin, γ-endorphin, DTαE, and DTγE are present in rat brain. These observations support the view that β-endorphin fragments such as α- and γ-endorphin and their des-tyrosine fragments are endogenous peptides in the CNS. In addition Burbach et al. (67) used this method to analyze the peptide fragments that are formed when β-endorphin is converted by proteolytic enzymes associated with SPM preparations. It appeared that α-endorphin, γ-endorphin, DTγE, and DTαE are formed from β-endorphin when a relatively crude preparation is used and with a purified SPM obtained by sucrose-density gradient centrifugation. Apparently the enzymes involved in the formation of these β-endorphin fragments in brain are located in the SPM. Furthermore it was recently shown that homogenous bovine brain enkephalin-degrading aminopeptidase catalyzed the hydrolysis of the NH_2-terminal tyrosine from Met-enkephalin and from α-, γ-, and β-endorphin, thus yielding the des-Tyr fragments of these endorphins (183). The rate of hydrolysis of α- and γ-endorphin was 30% of that of Met-enkephalin, whereas β-endorphin was hydrolyzed at a much lower rate.

The accumulation of peptide fragments during digestion of β-endorphin by SPM-associated enzymes in vitro appeared to be highly pH dependent (67). After the formation of γ-endorphin, DTγE accumulated maximally at neutral pH (pH 6.7), whereas α-endorphin and DTαE accumulated preferentially at a lower pH (pH 5.9). The preferential formation of these two types of ligands with opposite effects indicates that the enzymes can be modulated so that the balance between γ- and α-type peptides is changed. Because this modulation in vitro was achieved by pH changes over a narrow, near-physiological range, the effect of experimental pH changes in vitro may mimic the modulation of physiological processes in vivo.

Thus β-endorphin may serve as a precursor molecule for neuropeptides with specific and distinct properties. It is conceivable that systems regulating the balance between the α- and γ-type endorphins play a significant role in the control of adaptive behavioral responses of the organism. Therefore the preferential conversion of β-endorphin into γ- or α-type endorphins may provide a biochemical basis for the hormonal regulation of adaptive behavior. Derangements in this regulatory system are probably an important factor in psychopathology.

XI. ELECTROPHYSIOLOGICAL AND MOTONEURON EFFECTS OF NEUROPEPTIDES DERIVED FROM PRO-OPIOCORTIN

A. Electrophysiological Effects of ACTH-Like Peptides

Effects of pituitary-adrenal system hormones on electrical activity of the brain have been reported in early clinical studies. These findings have stimulated experimental investigations of this subject. Torda and Wolff (443)

showed that a single administration of ACTH induced an increase in electroencephalogram (EEG) voltage, occasional spiking, paroxysmal runs of low frequency, high-voltage waves, and lowered convulsion threshold for pentamethylenetetrazol. Adrenocorticotropin appeared to be active in intact rats and also in hypophysectomized and adrenalectomized rats. The peptide-induced hyperexcitability of the brain therefore did not depend on the presence of the adrenal cortex. Another parameter used in the study of electrophysiological actions of ACTH is the electroconvulsive threshold (EST). This measures the appearance of tonic-clonic seizures, which develop at much higher electroshock intensity than the electrocortical spiking measured by Torda and Wolff (443). Woodbury et al. (521) found that ACTH slightly increased EST and prevented the substantial increase in EST as a result of deoxycorticosterone treatment. Wasserman et al. (471), on the other hand, reported that ACTH caused a reduction in the threshold for minimal clonic electroshock seizures in young rats. This effect also occurred independent of the adrenal cortex. Furthermore, after microinjection of $ACTH_{1-24}$ into the neocortex or hippocampus of anesthetized rats, waves of spreading depression were elicited in both structures (206). This effect was interpreted as being related to excessive grooming, penile erection, and SYS, but the significance of these findings is not yet clear.

Krivoy and co-workers (249, 251) were the first to demonstrate a neuromodulatory role of β-MSH using the segmental reflex in cats. In Nembutal-treated and decerebrate cats, β-MSH potentiated the amplitude of submaximal monosynaptic potentials from the ventral root after dorsal root stimulation. In the frog $ACTH_{1-24}$ had no direct effect on either the segmental reflex or on the monosynaptic ventral root response of the isolated spinal cord. However, both β-MSH and $ACTH_{1-24}$ effectively antagonized the decremental effect of morphine on these preparations (250, 535). Nicolov (321) studied the influence of ACTH in dogs with electrodes chronically implanted in their spinal cords. After a latent period ACTH increased electrical activity of the spinal cord. Cortisol induced similar changes, indicating that this effect of ACTH was mediated through the adrenal cortex. Another spinal effect of ACTH was found by Korányi and Endröczi (241), who reported that ACTH inhibited the polysynaptic flexor reflex in the rabbit.

Adrenocorticotropin and related peptides also affect brain electrical activity at the supraspinal level. Dyster-Aas and Krakau (102) found that rabbits given α-MSH exhibit slow-wave activity and no cortical activation in response to arousing stimuli. Korányi et al. (242) similarly found that conditioned EEG arousal in the rabbit was suppressed by ACTH, whereas Kawakami et al. (230) found an increased high-frequency activity in the amygdaloid nucleus, an increase in the θ-type (4–12 Hz) of activity in the reticular formation, and a decrease of this activity in the hippocampus. Furthermore the peptide induced a burst of spindles with a relatively short latency in the septal area and in the somatomotor cortex (241). After the administration of α- or β-MSH in the rat, Sandman et al. (383) found an

increase in high-voltage, slow-wave activity in the occipital cortex that resembled limbic activity. A similar effect of α-MSH on EEG activity recorded from the basal preoptic and hypothalamic areas was found in the frog (91). After systemic injection of ACTH no visible alterations in cortical EEG of rats were found (343), but Sawyer et al. (390) observed a cortical desynchronization after ACTH and an increase in multi-unit activity with a relatively short latency in the basal hypothalamus and dorsolateral thalamus. This increased unit activity was followed by a depression of long latency. The depression was absent in adrenalectomized rats but could be mimicked by dexamethasone.

Using iontophoretic application of $ACTH_{1-24}$ in hypothalamic and midbrain nerve cells, Steiner et al. (412) showed an increase in single-unit activity; the glucocorticosteroid dexamethasone, however, had the opposite effect. Systemic administration of ACTH increased hypothalamic single-unit activity in the intact rat (90), and increased unit activity was also found in the dorsal hippocampus of both intact and hypophysectomized rats. Corticosterone had the opposite effect (343). Baldwin et al. (18) found an increase in multiunit activity in the region of the lateral preoptic–diagonal band of Broca and the periventricular preoptic area after ICV $ACTH_{1-24}$ but not $ACTH_{4-10}$ in the rabbit. An increased multiunit activity was also found in the mesencephalic reticular formation after administration of ACTH in the cat, although a slight decrease was found in the medial preoptic area (239). In one study (240) a decreased multiunit activity was found in the mesencephalic reticular formation, the midline thalamic nucleus, and the hypothalamus during attentive behavior and paradoxical sleep and also in response to sensory stimulation. This effect was found both in intact and adrenalectomized cats. Adrenocorticotropin (but not $ACTH_{4-10}$ or $ACTH_{4-7}$) increased the firing rate of brain stem cells (133). This response was markedly reduced by [D-Phe^7]$ACTH_{4-10}$. Responses to acetylcholine or L-glutamate were unaffected by the D-peptide. Most studies suggest that ACTH has an excitatory effect on unit activity, but no conclusions can be drawn in view of the opposite effects of corticosterone on the same parameter (343) and the inactivity of the fragment $ACTH_{4-10}$. Therefore to correlate unit activity of ACTH with its behavioral actions, more studies are needed with other behaviorally active, but endocrinologically inert, neuropeptides.

Studies with rhythmic slow activity [RSA or θ-activity (453)] used as a parameter for electrophysiological effects of ACTH suggest that effects of ACTH and related peptides on avoidance behavior correlate rather well with alterations in this parameter. Gray (157) showed that septal driving of hippocampal θ-activity with a frequency of 7.7 Hz decreased the resistance to extinction of a rewarded runway response. Effects of $ACTH_{4-10}$ on hippocampal θ-activity were reported by Urban et al. (451) in the dog. These authors found a shift in the dominant frequency of hippocampal RSA during prestimulus "facing" periods of a conditioned food-reinforcement operant response. The peptide shifted the dominant frequency in the direction of

lower frequencies without affecting the stabilized behavior. In contrast $ACTH_{4-10}$ induced a shift in the dominant frequency of hippocampal and posterior thalamic RSA from 7.0 to 7.5 Hz and increased the appearance of 7.5- to 9.0-Hz components when RSA was evoked by electrical stimulation of the reticular formation of the freely moving rat (452). Because a similar shift to higher frequencies was produced by an increase in stimulus strength, it was suggested that the peptide increases the excitability state in limbic-midbrain structures (422). In rats $ACTH_{4-10}$ also accelerated the hippocampal RSA produced during paradoxical sleep. [D-Phe^7]$ACTH_{4-10}$ had an opposite effect on RSA induced by paradoxical sleep and slowed down the rate of rhythmicity of this activity (454). Activation of the reticular activating system (which induces cortical arousal) is accompanied by θ-activity in the hippocampus (160), and the frequency of the θ-rhythm depended in part on the intensity of the stimulation of the midbrain reticular formation (236). These observations may support the hypothesis that $ACTH_{4-10}$ increases the state of arousal in the limbic-midbrain system and thereby increases the probability that specific behavioral responses are induced (490). This view is supported by studies on visually evoked potentials in the rat (517) in which the amplitude of the averaged visually evoked response afterdischarge in cortical area 17 was significantly reduced by $ACTH_{4-10}$ and also by [D-Phe^7]$ACTH_{4-10}$. These findings were interpreted to indicate that ACTH-like peptides activate the CNS vigilance-regulating system.

B. ACTH-Like Peptides and Motor Systems

Adrenalectomized rats that were stressed or treated with $ACTH_{1-39}$ appeared to have an enhanced muscular performance and reduced fatigue of the motor unit in situ (416, 418). The effect of ACTH was also seen when these animals were hypophysectomized and appeared to be extra-adrenal, since α-MSH and $ACTH_{4-10}$ had similar effects on peripheral motor systems (416, 421). The increase in muscle potential and contraction amplitude after adrenalectomy is presumably a result of high levels of circulating ACTH (187). This view is supported by findings in hypophysectomized animals (417, 418). A decrease in amplitude of the action potential was observed during repetitive stimulation of the gastrocnemius or extensor digitorum longus muscles. Concomitant with this decrease in amplitude a fall in height and a prolongation of the duration of the isometric twitch were observed, which were due to an increased contraction time and half-relaxation time of this parameter (420). Administration of $ACTH_{1-39}$ and also α-MSH and $ACTH_{4-10}$ increased muscle contraction and muscle action potential amplitude and reduced fatigue of the motor unit in hypophysectomized rats. [D-Phe^7]$ACTH_{4-10}$, which has an effect on avoidance behavior opposite to that of $ACTH_{4-10}$ (162), was ineffective. All peptide action was eliminated when the curarized muscle was directly stimulated and when the distal stump was

cut just prior to stimulation. Thus the influence of ACTH and its fragments seemed to be directed to the perikaryon of the motoneuron itself rather than to its peripheral components, again suggesting that ACTH acts directly on the nervous sytem.

Intra-arterial injection of $ACTH_{4-10}$ increased miniature end-plate potentials (MEPPs) frequency in hypophysectomized rats. Postsynaptic events such as MEPP amplitude, duration, interval, and rate of rise were unaffected (420). This is in accord with findings by Birnberger et al. (31), who used in vitro phrenic nerve-diaphragm preparations of intact rats. These authors found that ACTH reduced the quantum content of end-plate potentials (EPPs); ACTH also increased the transmission failure rate and the frequency of MEPPs. These results were interpreted as a presynaptic effect. Injection of $ACTH_{4-10}$ increased only MEPP frequency, whereas quantum content and failure rate of EPPs remained unchanged.

In studies on single neurons of the cat spinal cord, Krivoy and Zimmerman (252, 253) found that β-MSH selectively facilitates the posttetanic recovery of α-motoneurons. The time interval required before the next spike potential could be evoked was shortened. This effect is slow in onset and of long duration. No change in synaptic delay time, rise time of the excitatory postsynaptic potential (EPSP), or resting membrane potential was observed, indicating that β-MSH had little effect on postsynaptic elements. Because Renshaw cells are not influenced by β-MSH, suppression of recurrent inhibition is not involved in the effect of β-MSH on motoneurons. However, a postsynaptic effect of $ACTH_{4-10}$ has been reported on synaptic transmission in the paravertebral sympathetic ganglion of the frog (523). Postsynaptic potentials evoked by electrical stimulation of preganglionic nerves were recorded by a sucrose-gap method. Fast EPSPs, which are mediated via nicotinic cholinergic synapses, were not affected by 10^{-6} M $ACTH_{4-10}$. Application of $ACTH_{4-10}$ in a concentration as low as 10^{-8} M for 60 min caused a marked augmentation of the amplitude of slow inhibitory postsynaptic potentials (IPSPs), which are presumably mediated by dopaminergic synapses. The increase in amplitude developed gradually after a latency of 60–90 min and outlasted the application of the peptide. In addition $ACTH_{4-10}$ at 10^{-6} M increased the hyperpolarizing response of the ganglion to exogenously added DA. There was no significant effect of $ACTH_{4-10}$ on the muscarinic cholinergic depolarizing response of the ganglion toward exogenous acetylcholine. The authors concluded that $ACTH_{4-10}$ specifically affected slow synaptic inhibition in the frog sympathetic ganglion, probably by acting on the postsynaptic membrane (523).

Strand and Smith (419) found that $ACTH_{4-10}$ also exerts a potent effect on motor systems in the immature rat. They used animals under 15 days of age and stimulated the extensor digitorum longus muscle; $ACTH_{4-10}$ increased the contraction amplitude and decreased the half-relaxation time. Strand and Smith (420) further studied the effect of $ACTH_{1-24}$ on functional and structural changes in nerve and muscle of normal and adrenalectomized

rats after crush denervation of the peripheral nerve of the foot. The movements of the denervated foot were tested by heat avoidance. Adrenalectomized rats treated with ACTH performed the normal movement considerably faster than saline-treated rats. This was attributed to more rapid regeneration of crushed axons. The number of large end plates increased, as did the frequency of preterminal branching in end plates. However, no effect of ACTH on muscle per se was observed. These findings were confirmed by Bijlsma et al. (30), who showed that $ACTH_{4-10}$ and also the potentiated $ACTH_{4-9}$ analogue Org 2766 possessed this effect.

C. Electrophysiological Effects of Endorphins

Most studies on the electrophysiological effects of the enkephalins and β-endorphin concern the typical opiatelike effects of these peptides. Accordingly a brief survey is given of relevant data only on the electrophysiological effects of the endorphins. The review by North (323) critically evaluates the data.

Opioid peptides have consistently been found to inhibit neural firing, and this effect is readily reversed by naloxone. The inhibition has been found both for enkephalins and β-endorphin, but the onset and offset appeared more rapid for enkephalins (323). Most studies have been performed in the rat. The spontaneous firing of single neurons has been monitored or the firing induced by glutamate or other transmitters. Enkephalin and more stable analogues as well as β-endorphin inhibited the firing rate of single neurons in the neocortex (122, 186, 320), the caudate putamen (122, 124), the nucleus accumbens (289), the thalamus (185, 320), the periaqueductal gray (122, 518), and the brain stem (58, 170, 320). The more stable analogues [D-Ala^2-]Met-enkephalin and [D-Ala^2]D-Leu^5-enkephalin had an action of longer duration (58). Remarkably in the cat, in which enkephalin also inhibits the firing rate of brain stem neurons, the effects were not readily reversed by naloxone (134, 518). Gent and Wolstencroft (134) and Wolstencroft et al. (518) used β-endorphin and Leu-enkephalin, which mainly had a depressant effect, whereas α-endorphin and Met-enkephalin had a depressant as well as an excitant action. In the rat naloxone-irreversible depression of neurons by enkephalin has also been reported (186).

Evoked responses of single neurons are also reduced by endorphins. Iontophoretic application of enkephalins inhibited the response of thalamic neurons to painful stimulation (185, 186). This effect could be antagonized by naloxone. When applied into the substantia gelatinosa of spinal cats, Met-enkephalin selectively inhibited the response of the lamina V cells of spinal cats to noxious stimuli. Weak inhibitory effects on spontaneous firing were also noted (96). These effects were antagonized by naloxone administered locally but not after systemic injection. In cell cultures of mouse spinal cord neurons, Leu-enkephalin depressed the response to iontophoretically applied glutamate (21).

Excitation by iontophoretically applied enkephalins has been observed in Renshaw cells (86) and in hippocampal neurons. Hippocampal single neurons are excited by enkephalin and β-endorphin (124, 184, 320, 527). These excitatory effects occur as the result of disinhibition, since enkephalin depressed the tonically active inhibitory interneurons, which probably contain GABA (526). This was confirmed by Nicoll et al. (319), who found that enkephalin excites hippocampal cells by blocking both spontaneous and evoked inhibitory potentials. Also feedforward and feedback inhibitory pathways were depressed by enkephalin. All these effects were reversed by naloxone. Such a disinhibition is a possible explanation for the nonconvulsive limbic epilepticlike activity found after administration of β-endorphin in nonanalgesic amounts (182). Leu- and Met-enkephalin (but not [D-Ala^2]Met-enkephalinamide) had similar effects (123). Finally, β-endorphin as well as β-LPH$_{61-69}$ caused a shift in the dominant frequency of hippocampal RSA to higher activities during paradoxical sleep episodes (490). Similar effects were found after ACTH$_{4-10}$ (452), although the influence of the endorphins appeared to last longer.

XII. MOLECULAR MODE OF ACTION OF NEUROPEPTIDES DERIVED FROM PRO-OPIOCORTIN

A. Introduction

From the behavioral studies described in the previous sections, it was concluded that neuropeptides derived from the pro-opiocortin molecule act directly on the nervous system. Research into the neurochemical mechanism of action of the neuropeptides has traditionally focused attention on the metabolism of proteins and nucleic acids. It was hypothesized that a peptide might change the transcription of DNA into RNA and/or the translation into protein. It was originally thought that "memory molecules" are thereby produced. The present notion is that changes in nerve cell protein synthesis will change the dynamic properties of the neuron [for review see Dunn and Gispen (98)].

Other studies have measured the effects of neuropeptides on the metabolism of neurotransmitters in the brain. In addition specific membrane receptors have been found for endorphins, and the multiplicity and specificity of the ACTH-CNS interactions suggest that specific ACTH receptors are also present in the brain. It is therefore conceivable that the neuropeptides affect processes in the nerve cell membranes and that these effects underlie the behavioral action of these substances. Some recent research into the neurochemical effects of the neuropeptides is reviewed.

B. Ribonucleic Acid Metabolism

The effects of neuropeptides on general cellular metabolism may be reflected in an altered metabolism of RNA and of protein synthesis.

In a series of experiments, Jakoubek et al. (207) studied the influence of ACTH on brain RNA metabolism. A single high dose of purified ACTH resulted in a transient decrease of uridine incorporation into mouse brain RNA. These findings were confirmed in rats by Schotman et al. (397). These authors found that injection of $ACTH_{1-24}$ also led to a small but significant decrease (−12%) in uridine incorporation of brain stem RNA. Cerebral and cerebellar RNA were also affected. The decrease in RNA labeling was accompanied by a transient decrease in brain stem RNA content 2.5 h after the injection of $ACTH_{1-24}$. In adrenalectomized rats (36 h after surgery) similar treatment stimulated (+40%) instead of inhibited the labeling of brain stem RNA, although brain stem RNA content was not decreased. Changes in uridine incorporation could not be attributed to differences in brain precursor uptake or to gross changes in precursor metabolism in brain tissue (145). Administration of $ACTH_{1-10}$ was ineffective in both intact and adrenalectomized rats. Chronically administered $ACTH_{4-10}$ did not affect the labeling and content of mouse brain RNA (352), and no effects of this treatment were found on the labeling of messengerlike and ribosomal brain stem RNA (394), and the content of brain stem polysomes (146). The corticotropic effect of $ACTH_{1-24}$ may be responsible for the differential action of this peptide in intact and adrenalectomized rats. The fact that $ACTH_{1-10}$ was ineffective in stimulating uridine incorporation into brain stem RNA in the various experimental studies (intact, adrenalectomized, hypophysectomized rats) may reflect a difference in information content between $ACTH_{1-10}$ and $ACTH_{4-10}$ and longer ACTH sequences such as $ACTH_{1-16}$, $ACTH_{1-24}$, and $ACTH_{1-39}$.

C. Protein Synthesis

Neuropeptides influence the synthesis of brain proteins [for review see Dunn and Gispen (97)]. The rate of incorporation of labeled amino acids into brain protein was decreased after hypophysectomy (463). When these hypophysectomized rats were treated during a 12-day period with $ACTH_{1-10}$, the incorporation of [^{3}H]leucine into proteins of the brain stem was normalized (363, 394). In subsequent experiments it appeared that chronic treatment of hypophysectomized rats with $ACTH_{1-10}$ interfered with overall protein synthesis rather than with certain particular protein species (366). Similar conclusions emerged from in vitro experiments. Both $ACTH_{1-10}$ (365) and $ACTH_{4-10}$ (272) stimulated the incorporation of leucine into protein of brain stem slices. Jakoubek et al. (207) showed that high doses of ACTH affected incorporation of [^{14}C]leucine into proteins of the nervous system of mice. Treatment with ACTH increased [^{14}C]leucine incorporation into brain protein in vivo (208), although a decreased [^{14}C]leucine incorporation was found in cortical slices (209). Reading and Dewar (352) showed that chronic $ACTH_{4-10}$ treatment of intact mice stimulated the incorporation of ^{14}C-la-

beled glycine and leucine into cerebral protein. Rudman et al. (379) demonstrated that a single injection of either ACTH or β-MSH increases the rate of [^{14}C]valine accumulation into mouse brain proteins in a period of 6–24 h after the intraperitoneal injection of the radioactive precursor. Flood and Jarvik (119), however, failed to find an effect of $ACTH_{4-10}$ and [D-Phe^7]$ACTH_{4-10}$ on [^{14}C]valine incorporation into mouse brain protein at several time intervals after injection of the peptides. The possibility that an altered brain uptake of amino acid after treatment with peptide (e.g., $ACTH_{1-10}$, ACTH, or α-MSH) might contribute to the observed changes in incorporation into brain proteins could be discarded (379, 396).

Structure-activity studies showed that $ACTH_{1-24}$ stimulates protein labeling in vitro similarly to $ACTH_{1-10}$ (367), whereas the sequence $ACTH_{11-24}$, which is behaviorally much less active, did not affect [^{14}C]leucine incorporation into brain proteins under both in vivo and in vitro conditions (367, 394). Chronic treatment with [D-Phe^7]$ACTH_{1-10}$ decreased the in vivo incorporation of [^{3}H]leucine into total brain stem protein of hypophysectomized rats (366, 394) but not of intact mice (348). Under in vitro conditions no effects of [D-Phe^7]$ACTH_{1-10}$ on [^{14}C]leucine incorporation were found (272, 366), but $ACTH_{4-10}$ was active in this respect.

Protein synthesis in pineal gland tissue in vitro also appeared sensitive to ACTH and related peptides. A biphasic dose-response curve was found for $ACTH_{1-24}$: the peptide had a stimulatory effect at 0.01–100 nM and an inhibitory effect at 10 μM. The effect of ACTH was not present after denervation of the gland, which may indicate that noradrenergic mechanisms were involved (395). Furthermore the effects of ACTH as well as of epinephrine and cyclic adenosine 3',5'-monophosphate (cAMP) were calcium dependent. Structure-activity studies showed that $ACTH_{1-16}$ was similar in action to $ACTH_{1-24}$, but $ACTH_{1-10}$ and $ACTH_{4-10}$ were inactive.

Effects on protein synthesis in cell-free brain systems have also been observed. Schotman et al. (395) found that $ACTH_{1-24}$ in doses of 0.01–0.1 μM stimulated the incorporation of [^{14}C]leucine or [^{14}C]phenylalanine into protein. At higher concentrations (100 μM) a pronounced inhibition occurred. The stimulatory effect of $ACTH_{1-24}$ was shared by $ACTH_{1-16}$, $ACTH_{1-10}$, and $ACTH_{4-10}$ but not by $ACTH_{11-24}$. On the other hand the inhibitory effect of high doses of $ACTH_{1-24}$ was also found for $ACTH_{11-24}$ but not for $ACTH_{1-10}$.

D. Neurotransmitters

1. ACTH and related peptides

Many reports have been published concerning the effects of neuropeptides on neurotransmitter metabolism in the brain, but the results remain confusing [for a recent review see Versteeg (462)]. Hökfelt and Fuxe (189) reported that ACTH increases the turnover of norepinephrine in the hy-

pothalamus, cortex, and other brain regions of intact rats. This could explain why hypophysectomy was found to cause a decreased epinephrine turnover (126, 189, 463, 479), whereas adrenalectomy was associated with an increased norepinephrine turnover in the brain (189, 211, 479). Changes in turnover of this neurotransmitter may therefore underlie the impaired avoidance behavior of hypophysectomized rats and the resistance to extinction of adrenalectomized animals (49, 483). The rate of extinction may be determined by norepinephrine turnover in the brain and controlled by a modulatory effect of ACTH on this turnover.

Some research has focused attention on the activity of enzymes involved in norepinephrine synthesis. Van Loon et al. (278) reported that tyrosine hydroxylase (TH) activity is increased in the hypothalamus, pons-medulla, and cerebellum but not in the striatum of hypophysectomized rats. Chronic treatment with a low dose of $ACTH_{1-24}$ normalized TH activity in the hypothalamus, and to a certain extent in the pons-medulla, but did not have such an effect in the cerebellum and striatum (278). Similarly it was found that ACTH treatment of hypophysectomized rats counteracted the hypophysectomy-induced decrease in dopamine β-hydroxylase activity of the hypothalamus and pons-medulla but not that of the cerebellum (278). In contrast to these findings with hypophysectomized rats, van Loon et al. (278) found no effect of $ACTH_{1-24}$ on regional TH activity in the brain of intact rats. In this respect the studies in which the effect of treatment with ACTH fragments was measured on regional TH activity in the brain are conflicting. Dunn et al. (100) initially reported that in mice treated for 3 days with $ACTH_{4-10}$, striatal TH activity was 50–60% higher than that of vehicle-treated animals. In a more extensive study, however, Dunn et al. (97) were unable to find a significant effect of the behaviorally potent ACTH analogue Org 2766 on TH activity in mouse striatum and in any of the other brain parts studied.

Catecholamine synthesis in whole brain and brain stem was increased after treatment with $ACTH_{4-10}$ for several days (466). This effect was absent in hypophysectomized or adrenalectomized rats. Similar findings were done in mice: $ACTH_{1-24}$, $ACTH_{4-10}$, and [D-Phe^7]$ACTH_{4-10}$ caused an increase in the conversion of [^{3}H]tyrosine into [^{3}H]dopamine but not into [^{3}H]norepinephrine (100, 196). After adrenalectomy $ACTH_{4-10}$ was ineffective (196).

Studies with α-MSH on α-methyl-*p*-tyrosine-induced catecholamine disappearance in the brain of intact and hypophysectomized rats failed to detect a correlation between effects on behavioral parameters and effects on norepinephrine turnover in the brain (241). In intact rats $ACTH_{4-10}$ was found to increase norepinephrine turnover in whole brain and in brain stem (461). [D-Phe^7]$ACTH_{4-10}$, which has an opposite effect on extinction of avoidance behavior, did not affect norepinephrine turnover. On the other hand Leonard (260) found that chronic administration of both $ACTH_{4-10}$ and [D-Phe^7]$ACTH_{4-10}$ caused a slight increase in whole-brain norepinephrine turnover. The results of these latter studies did not favor the hypothesis that

a simple relationship exists between brain norepinephrine turnover and the avoidance performance of rats. With respect to the MSH-like peptides, neither α- nor β-MSH affected the conversion of [^{3}H]tyrosine into ^{3}H-labeled catecholamines in mouse brain (100, 196).

Intracerebroventricular administration of relatively high doses of $ACTH_{1-24}$ and $ACTH_{4-10}$ caused an increase in the rate of disappearance of intraventricularly administered [^{3}H]norepinephrine from the hypothalamus, hippocampus, and cortex of adrenalectomized rats (106, 108). Implantation of $ACTH_{4-10}$ in the region of the locus ceruleus, but not in the septal region or in the medial forebrain bundle at the level of the posterior hypothalamus, also caused an increase in the disappearance of [^{3}H]norepinephrine from hippocampus, hypothalamus, and cortex (107). These results implicate an effect of ACTH and analogues at the level of the cell bodies of the dorsal noradrenergic bundle, which might be related to the effects of these peptides on behavior.

The dopaminergic nigrostriatal system may be involved in ACTH-induced excessive grooming (497). Apparently ACTH may exert its effect by modulating neurons in the substantia nigra (85). These data agree with observations by Dunn et al. (100) and Iuvone et al. (196), who found an increased striatal dopamine turnover after $ACTH_{1-24}$ and [D-Phe^7]$ACTH_{4-10}$.

Effects of ACTH-like peptides on other neurotransmitters have also been found. After ICV administration, $ACTH_{1-24}$ and α-MSH in a dose of 10 μg/rat induced an increase in hippocampal acetylcholine turnover but not in the cortex, striatum, and diencephalon; α-MSH also affected the turnover of acetylcholine in the brain stem (520). It was suggested that the effects of $ACTH_{1-24}$ and α-MSH on excessive grooming, stretching, and yawning might be related to the effect of these peptides on hippocampal acetylcholine turnover. Interestingly Org 2766, which does not induce SYS nor excessive grooming, also has no effects on acetylcholine turnover in the hippocampus (520). Based on these findings and on the observation that intraseptal injection of $ACTH_{1-24}$ and α-MSH failed to affect hippocampal acetylcholine turnover, Wood et al. (519) suggested that these peptides exert their stimulating effect on the turnover of acetylcholine in the hippocampus by activating receptors located in this structure, although the site of action might be the septal area.

Data on the effects of ACTH and related peptides on the metabolism of serotonin in the brain are few and controversial. Chronic administration of $ACTH_{4-10}$ or [D-Phe^7]$ACTH_{4-10}$ decreased the concentration and turnover of serotonin (260). Subsequent studies showed that similar treatment with α-MSH increased the conversion of [^{3}H]tryptophan into [^{3}H]serotonin in rat cortex but not in other brain regions. Spirtes et al. (409), however, found no effect of α-MSH on serotonin concentration and serotonin turnover in the brains of intact rats, whereas serotonin accumulation after pargyline was decreased in the cortex of hypophysectomized rats after treatment with α-MSH. Acute effects of $ACTH_{1-24}$ were observed on serotonin concentrations of the hypothalamus, mesencephalon, and hippocampus (433). Although some

recent data suggest that the effects of [D-Phe^7]ACTH$_{4-10}$ and ACTH$_{4-10}$ on hippocampal serotonin levels do correlate with changes in passive avoidance behavior, it can be concluded that there is no convincing evidence for the existence of a relationship between influences of ACTH-like peptides on serotonin metabolism in the brain and the effects of these peptides on behavior.

2. Endorphins and related peptides

Numerous studies have investigated the possible effects of enkephalins on the metabolism of several neurotransmitters [for a recent review see Versteeg (462)]. Catecholamine release is generally reduced after the ICV administration of the enkephalins or analogues that are more resistant to enzymatic degradation. Met-enkephalin was shown to decrease the release of [^{3}H]norepinephrine from slices of rat occipital cortex that was evoked by electrical field stimulation or high potassium concentrations (432). This effect was blocked by naloxone but not by an α-adrenergic blocker. It was concluded that one function of endogenous opioid peptides might be the presynaptic inhibition of central noradrenergic or dopaminergic neurotransmission. The primary effect of the enkephalins may result from activation of opioid receptors localized presynaptically on dopamine terminals in the striatum. This would then cause a decrease in dopamine release, which accounts for the decrease in dopaminergic transmission observed immediately after their administration. A secondary effect would be an increased DA synthesis due to decreased feedback inhibition of presynaptic DA receptors. This has been demonstrated by an increase in various parameters that estimate DA synthesis (5, 70, 347, 398).

Much less is known about effects of enkephalins on DA metabolism in other brain areas. In a study by Versteeg et al. (464) in which Met-enkephalin was given in much lower amounts than needed to induce analgesia, the disappearance of norepinephrine was measured after treatment with α-MPT. It was found that norepinephrine disappearance was enhanced in the medial preoptic nucleus and the central amygdaloid nucleus and decreased in the ventral gray and the A_2 region of the nucleus tractus solitarii. Dopamine disappearance was increased in the arcuate nucleus and the dorsal central gray. Thus catecholamine transmission may be affected in more than one way, and such effects may show up only if the methods used to determine neurotransmitter activity in small brain parts are sufficiently sensitive.

Reports on the effect of enkephalins on acetylcholine and serotonin neurotransmission are much fewer than those on catecholamines. Enkephalins reduce the release of acetylcholine from hippocampal slices (427) and from cortex (212). The situation in the striatum is more complicated. Influences in this brain part may be due to the inhibitory effect of the enkephalins on dopaminergic neurons, which inhibit cholinergic interneurons (467). Whole-brain serotonin metabolism seems to be enhanced after ICV enkephalin administration (4, 5, 282).

Effects of β-endorphin on catecholamine metabolism have been mainly studied in the striatum. After intracisternal administration this peptide increased the DA turnover in a naloxone-reversible way (276). Development of tolerance to intracisternal β-endorphin administration was associated with a return to a normal DA turnover in the striatum (278). The effects of β-endorphin in other brain regions may be either stimulatory or inhibitory, depending on the brain parts studied (462); the tuberoinfundibular dopamine system seems particularly sensitive to β-endorphin. The notion that opioid receptors on this system reduce the release of DA (125) was substantiated by the finding that ICV β-endorphin markedly reduced the DA concentration in pituitary stalk plasma (166). This effect was prevented by naloxone pretreatment.

Garcia-Sevilla et al. (130) studied the accumulation of dopa after inhibition of the enzyme dopa decarboxylase. They found that ICV β-endorphin significantly increased the concentration of dopa in striatum, limbic structures, and cortex, which may indicate an enhanced catecholamine synthesis. Versteeg et al. (465) found that low nonanalgesic doses of ICV-administered β-endorphin had no effect on α-MPT-induced disappearance of DA from either the caudate nucleus or the nucleus accumbens. It was also found that low doses of ICV-administered β-endorphin caused a decrease in α-MPT-induced disappearance of norepinephrine from the ventral part of the nucleus reticularis medulla oblongata and of DA in the lateral septal nucleus. Both amines were reduced in the rostral part of the nucleus tractus solitarii. The disappearance of DA was increased in the medial septal nucleus and the zona incerta. The effects of α-endorphin on the α-MPT-induced disappearance of catecholamines were much more widespread throughout the brain than those of β-endorphin. Moreover α-endorphin in all instances decreased the norepinephrine and DA disappearance. Interestingly, Versteeg et al. (465) found that DTγE affected DA disappearance from the paraventricular nucleus and the zona incerta in a direction opposite to that induced by α-endorphin. A similar increase in DA disappearance is found more widespread in the brain after the classic neuroleptic haloperidol. In fact Weinberger et al. (478) found that haloperidol affects the in vitro conversion of [^{14}C]tyrosine into [^{14}C]dopamine by caudate slices 1 h after in vivo administration. The same dose of DTγE, however, failed to do so. Perhaps the caudate nucleus is not the brain region where DTγE acts or the dose used after systemic administration was not sufficient to elicit such effects.

More clear-cut effects were noted on the turnover of acetylcholine. After ICV administration in analgesic amounts, β-endorphin caused a decrease in the turnover of acetylcholine in the hippocampus, nucleus accumbens, globus pallidus, and cortex but not in the caudate nucleus (309). In a nonanalgesic dose, α-endorphin did not affect acetylcholine turnover in any of the brain regions studied (309). Naltrexone completely prevented both the analgesic action and the effect of β-endorphin on acetylcholine turnover (309). In subsequent experiments Moroni et al. (310) found that intraseptal administration of β-endorphin reduced the turnover of acetylcholine in the hippocampus

but not in the striatum or cortex. The effect of intraseptal β-endorphin on hippocampal acetylcholine turnover was prevented by naltrexone. On the basis of these data it was concluded that opioid agonists modulate hippocampal acetylcholine turnover via opioid receptors in the septum (310, 311). Thus the activity of acetylcholine-containing neurons projecting from the septum to the hippocampus may be regulated by peptidergic neurons impinging on the cell bodies of the cholinergic neurons in the septum (310, 311). Moroni et al. (309) and Botticelli and Wurtman (52) found significant elevations of hippocampal acetylcholine levels after the ICV administration of β-endorphin. The maximum effects were found 30 min after peptide injection. The choline concentration was not changed, whereas naloxone antagonized the effects of β-endorphin on acetylcholine levels in the hippocampus. The conclusion that acetylcholine utilization in the hippocampus is decreased is in keeping with the suggestions of Moroni et al. (311).

The few studies on brain serotonin point to an increase in the turnover of this transmitter. Intracerebroventricularly administered β-endorphin caused an increase in serotonin accumulation in limbic structures, striatum, diencephalon, and lower brain stem but not in the cortex (13, 130, 282). Van Loon and de Souza (277) concluded on the basis of changes in serotonin and 5-hydroxyindoleacetic acid concentrations that β-endorphin decreases the pargyline-induced accumulation of serotonin in various brain parts. This effect, particularly in the brain stem and hypothalamus, was greater after repeated injections (278). This has also been found for the turnover of GABA. According to Moroni et al. (312), activation of opioid receptors located on short GABA-containing neurons in the caudate nucleus causes an increase of GABA turnover in the globus pallidus. This is due to a decrease in GABAergic inhibition elicited by short GABA neurons on the long GABA neurons linking the caudate nucleus to the globus pallidus. Perez de la Mora et al. (342) found evidence that β-endorphin increases GABA turnover also in the caudate nucleus.

In conclusion, the confusing and apparently contradictory results obtained so far may be the results of the different species, brain regions, methods, peptides, and dose levels used in these studies. The effect of these peptides on neurotransmitter metabolism may reflect indirect consequences of peptide action, however, whereas the direct influence of these principles may be on enzymatic processes in the nerve cell membrane.

E. Cyclic Nucleotide Metabolism

1. ACTH and related peptides

In the second-messenger concept of Sutherland and Robinson (428), interaction of a hormone (first messenger) with its membrane receptor activates the enzyme adenylate cyclase, giving rise to an increased level of cAMP

in the cell. This substance acts as an intracellular second messenger and mediates the hormone effect by activation of protein kinases (see sect. XIIF).

Apparently cAMP was involved in the process of ACTH-induced steroidogenesis in the adrenal cortex and of lipolysis in the fat cell (109, 171). In addition some data favoring such a role for cAMP in the brain have also been reported. Several authors were unable to detect an effect of ACTH on adenylate cyclase activity in cell-free preparations of rat brain tissue (68, 468) or rat cerebral cortex slices (119). On the other hand indirect indications that ACTH-like peptides might affect brain cyclic nucleotide levels in vivo were presented by Rudman and Isaacs (376, 378). They showed that intrathecal injection of microgram quantities of ACTH or β-MSH in rabbits increased the cAMP but not the cyclic guanosine 3′,5′-monophosphate (cGMP) concentration in cerebrospinal fluid. This increase in cAMP may have originated from ACTH-sensitive adenylate cyclase located in circumventricular organs (377). In a preliminary study, Christensen et al. (78) showed that chronic treatment with α-MSH increased the level of cAMP in the occipital cortex of intact as well as of hypophysectomized rats. Similar treatment left the level of cGMP unaltered (410). Wiegant et al. (498) obtained direct evidence that NH_2-terminal fragments of ACTH influence the cAMP accumulation in rat brain using three different approaches: adenylate cyclase activity was determined in broken-cell preparations and in slices from posterior thalamus and neostriatum, and cAMP levels were measured in vivo (498, 499). In broken-cell preparations, $ACTH_{1-24}$ had a biphasic effect on the activity of adenylate cyclase: it stimulated in micromolar concentrations, but it inhibited adenylate cyclase in concentrations 10–30 times higher. The basal activity and the ACTH effect appeared sensitive to Ca^{2+}. Furthermore an involvement of DA receptors and opiate receptors could be excluded. Interestingly the structure-activity relationship of the ACTH-like peptides on adenylate cyclase activity in vitro and the effect on excessive grooming behavior in vivo were the same (148, 500). It appeared that 0.1 mM $ACTH_{1-16}NH_2$ and $ACTH_{4-7}$ also inhibited adenylate cyclase activity, whereas $ACTH_{11-24}$, $ACTH_{1-10}$, $ACTH_{4-10}$, [D-Phe^7]$ACTH_{1-10}$, and [D-Phe^7]$ACTH_{4-10}$ were inactive. Although [D-Phe^7]ACTH analogues exert marked effects in various behavioral paradigms, their mechanism of action seems to differ from that of the natural all-L peptides (45, 48, 496).

In slices of posterior thalamus and neostriatum, $ACTH_{1-24}$ appeared to stimulate the activity of adenylate cyclase. The effect was rapid and of short duration. Finally, intraventricular injection of $ACTH_{1-16}NH_2$ in rats resulted in an increase in septal cAMP concentration. No effect was observed in other brain regions (498). These data suggest that ACTH-like peptides may be involved in brain cAMP metabolism.

2. *Endorphins and related peptides*

Most studies have failed to demonstrate direct effects of endorphins and enkephalins on basal adenylate cyclase activity in broken-cell preparations

or slices of brain tissue (74, 80, 83, 198, 434, 502). Motomatsu et al. (313), however, found that β-endorphin slightly inhibited the basal adenylate cyclase activity in homogenates of rat striatum. In addition this peptide was shown to inhibit adenylate cyclase activity in membrane preparations from rat cortex and brain stem, whereas Met-enkephalin stimulates the enzyme in the brain stem and inhibits it in the cortex (516). Naloxone antagonized both effects of Met-enkephalin. Furthermore inhibitory actions of opiates and opioid peptides have repeatedly been described in various adenylate cyclase preparations stimulated by prostaglandin E or biogenic amines (83, 313, 447, 469, 502). These effects could be antagonized by naloxone and were stereospecific.

In striatal slices, opiates and enkephalins not only lowered the prostaglandin E-stimulated cAMP accumulation (175) but also slightly inhibited basal adenylate cyclase (306). This effect was paralleled by a massive increase in cGMP formation, and the effects on both cAMP and cGMP were stereospecific and reversible by naloxone (306). The studies cited above indicate that opiates and endorphins inhibit the formation of cAMP in broken-cell preparations or slices of brain tissue.

F. Protein Phosphorylation

1. ACTH and related peptides

Changes in the phosphorylation of membrane proteins may govern the ion permeability of the neuronal membrane (178) or change the enzyme activity of such phosphoproteins (246). Protein phosphorylation may therefore be a key process in the regulation of the functional activity of the neuronal membrane and thus of the neuron.

Recently the phosphorylation of brain SPM proteins has been found to be sensitive to ACTH. In vitro $ACTH_{1-24}$ dose-dependently inhibited the endogenous phosphorylation of at least five proteins in an SPM preparation (531) through a direct action on the activity of membrane-bound protein kinase(s) (534). Interestingly this effect was not mediated by cAMP, since this nucleotide stimulated the phosphorylation of different membrane proteins. Structure-activity studies with ACTH-like peptides on endogenous phosphorylation of one particular SPM protein (B-50) showed that $ACTH_{1-24}$ and $ACTH_{1-16}$ were equally active and that $ACTH_{5-18}$ and $ACTH_{1-13}$ possess considerable activity. The sequences $ACTH_{11-24}$, $ACTH_{1-10}$, and $ACTH_{4-10}$ were inactive (534). This structure-activity relationship is very similar to that found for the induction of excessive grooming in vivo (148) and found for the inhibition of adenylate cyclase activity in broken cells (498) and for effects on polyphosphoinositide metabolism in vitro (214; see 213). Experiments on in vivo injection of ACTH and subsequent in vitro assay of endogenous phosphorylation of SPM proteins showed a dose-de-

pendent increase in phosphorylation of the same five SPM proteins. An increased in vitro phosphorylation under the experimental conditions used may be the result of in vivo inhibition of phosphorylating activity (533).

The ACTH-sensitive protein kinase and its substrate protein B-50 have been isolated from SPM as an enzyme-substrate complex. Further purification yielded an isolated protein kinase with a molecular weight of 71,000 and an isoelectric point of 5.5. The B-50 substrate protein had a molecular weight of 48,000 and an isoelectric point of 4.5. The enzyme was not sensitive to cAMP, but the presence of calcium was essential for B-50 protein phosphorylation. The concentration of $ACTH_{1-24}$ at which the effect was half-maximal was 5×10^{-6} M. Interestingly on proteolytic breakdown the B-50 protein yielded a small basic polypeptide that appeared to mimic the effect of $ACTH_{1-24}$ on B-50 phosphorylation and induction of excessive grooming behavior (532).

Attempts were made to localize the ACTH-sensitive B-50 protein in brain tissue: antibodies were raised against a partially purified B-50 protein preparation (327, 328). The immunohistochemical localization of B-50 was studied in sections of rat brain cerebellum and hippocampus. In agreement with the presumed synaptic origin of B-50, the antiserum reacted with tissue components in those parts of both brain regions that were rich in synaptic contacts. In contrast the white matter and cell perikarya did not contain specific immunostaining. The staining pattern was similar to that found by others using synaptic antigens (288) and suggests that at the cellular level there is a restricted localization of the B-50 protein in the synaptic region but that at the brain regional level the protein seems ubiquitous. Recent evidence suggests that the B-50 protein and its kinase may have an important role in membrane function by virtue of their enzyme activity in phosphoinositide metabolism (220).

2. *Endorphins and related peptides*

There have been few reports on effects of endorphins and enkephalins on protein phosphorylation, but some positive evidence has been obtained. Using an approach like that applied in the studies on ACTH-induced changes in membrane phosphoproteins, Davis and Ehrlich (87) reported that both Met-enkephalin and Leu-enkephalin inhibited the endogenous phosphorylation of two protein bands with apparent molecular weights of 47,000 and 15,000–20,000. When synaptosomal preparations had been preincubated with sodium ions or naloxone under conditions that promote dissociation of (endogenous) opiate agonists from the receptors, an enhanced phosphorylation of these proteins in particular was observed (87, 104). In a preparation so treated, β-endorphin inhibited the phosphorylation in a concentration of 10 nM. Evidence was presented suggesting that this peptide-induced inhibition was caused by interaction with stereospecific opiate-binding sites and that

one of these phosphoproteins is part of the opiate-receptor complex (105). It was hypothesized that phosphoproteins may control the recognition and/or affinity of opiate binding sites. In addition enkephalins have been found to affect protein phosphorylation in subcellular fractions obtained from rat hippocampus (20). Met-enkephalin and Leu-enkephalin seemed to enhance phosphorylation of one protein band with an apparent molecular weight of 50,000. Enkephalin-induced changes in phosphorylation could be mimicked by endorphin and could be completely suppressed by addition of naloxone to the medium. These results and the ineffectiveness of [des-Tyr^1]Met-enkephalin, a peptide without affinity for the opiate receptor (121), suggested that the effect on membrane phosphoproteins may be mediated by opiate receptors. Such a mechanism is in line with that proposed by others (104, 326). The enhancement found in the in vivo/in vitro approach (19) may not be at variance with the data on the inhibitory effect of enkephalin on protein phosphorylation in vitro as reported by Ehrlich et al. (87, 104). Furthermore, when Met-enkephalin was added to a hippocampal membrane fraction, a marked inhibition of phosphate incorporation into the 50,000-M_r band was observed (87). Although Davis and Ehrlich (87) point out similarities in the effects of enkephalins and ACTH, there are a number of differences. The 50,000-M_r band that is affected by enkephalin in hippocampal slices is not the B-50 protein (48,000 M_r), which is sensitive to ACTH. In addition the B-50 protein kinase activity was only very slightly affected by β-endorphin. The only endorphin that inhibits the B-50 protein kinase is dynorphin$_{1-13}$, a peptide recently isolated by Goldstein et al. (154).

G. Membrane Phospholipid Phosphorylation

Not only phosphorylated proteins (sect. XII*F*) but also membrane phospholipids have been implicated in the regulation of membrane permeability and synaptic transmission in neurons (176, 177, 301). Binding of a variety of agonists (hormones, neurotransmitters, etc.) to their respective receptors would initiate the hydrolysis of membrane polyphosphoinositides, followed by the influx of calcium. This ion is thought to act as a second messenger, thereby activating the target cells. To investigate whether ACTH-brain cell membrane interactions could involve such a mechanism, Jolles et al. (214, 218, 220) measured the phosphoinositide phosphorylation in synaptosomal fractions of rat brain in the presence of neuropeptides. The amount of label recovered in phosphatidylinositol (PI), phosphatidylinositol 4-phosphate (DPI), phosphatidylinositol 4,5-diphosphate (TPI), and phosphatidic acid (PA) were measured. After the demonstration that ACTH$_{1-24}$ inhibits the phosphorylation of these phospholipids in intact synaptosomes (218), experiments were carried out with lysed membrane fractions from synaptosomal origin (214, 219). Adrenocorticotropin appeared to stimulate the formation of TPI and to inhibit both the production of PA and the protein phosphor-

ylation in this fraction. As expected, Ca^{2+} was crucial for the hormone effects (219): the stimulatory effect of ACTH on TPI formation was maximal in the absence of Ca^{2+}, and 1 mM of this ion completely abolished the effect of the peptide.

In structure-activity studies (214), a direct correlation was found between the effects of ACTH fragments on PA and TPI formation and those obtained on B-50 phosphorylation in SPM. The effect of the peptide on TPI formation (stimulation) and PA formation (inhibition) decreased in the order $ACTH_{1-24} > ACTH_{5-18} > ACTH_{1-16} > ACTH_{1-13}$; $ACTH_{1-10}$ was ineffective. Shortening of the ACTH molecule at the amino terminus from $ACTH_{1-16}$ to $ACTH_{7-16}$ replaced the stimulatory effects on TPI formation for a similar effect on DPI. Furthermore loss of the doublet of lysine residues at the positions 15 and 16 of ACTH abolished the peptide effect on PA formation. Of the endorphins tested, only β-endorphin influenced the lipid metabolism. This peptide inhibited PA formation. The smaller endorphins, including the enkephalins, were inactive (214).

With respect to the mechanism of action, the evidence described so far suggested that a relation might exist between protein phosphorylation and polyphosphoinositide metabolism. Direct evidence supporting this notion was also obtained: the protein-phosphorylating system that is sensitive to ACTH was isolated from the membrane. It appeared to have both protein- and lipid-phosphorylating activity (220); ACTH and also β-endorphin (213) affected this activity. An important finding was that the amount of lipid phosphorylation depended on the degree of phosphorylation of the B-50 protein (220). The available evidence suggested that the B-50 protein might be a regulatory subunit of the DPI kinase that catalyzes the formation of TPI. An autophosphorylation of this enzyme (i.e., B-50 phosphorylation) would thereby determine the lipid kinase activity, and ACTH interferes with this process. This suggested that B-50, its kinase, and the relevant lipid molecules might be present in the membrane as a functional complex. The phosphorylation of protein and phospholipid thus would constitute two subsequent steps in the chain of events between hormone-receptor coupling and the intracellular response.

Both DPI and TPI are very potent chelators of calcium and magnesium ions (176, 177, 301). They interact strongly with proteins, and it has been proposed that the polyphosphoinositide may carry the negative potential of the membrane (442). Thus a change in the relative amounts of PI, DPI, and TPI in the synaptic membrane, brought about by an ACTH-induced inhibition of B-50 protein phosphorylation, may induce changes in membrane characteristics and in the amount of calcium and magnesium bound to the membrane. The close correlation between ACTH structure-activity relationships on in vitro parameters (inhibition of B-50 phosphorylation, stimulation of TPI, and inhibition of PA formation) and excessive grooming behavior suggests that such a neurochemical mechanism may underlie the effects on grooming behavior induced by these peptides.

H. Neurochemical Action of ACTH and Related Peptides

Adrenocorticotropin and related peptides affect neurotransmitter metabolism. Although the results are confusing, changes in neurotransmitter activity by neuropeptides derived from the pro-opiocortin molecule suggest a neuromodulatory influence of these neuropeptides. The way in which these changes are brought about is unknown but may involve processes in the cell or in the cell membrane. It has been shown that neuropeptides related to ACTH affect RNA and protein metabolism. Other studies reported above show that ACTH-like peptides, and also endorphins, have direct in vitro effects on some membrane processes such as adenylate cyclase activity, protein phosphorylation, and polyphosphoinositide metabolism. Adrenocorticotropin has been shown to interact specifically with membrane lipids (12, 392), whereas extensive studies by Loh and co-workers (275, 524) have demonstrated that membrane lipids can be specific receptors for opioids such as β-endorphin.

A functional relationship between the peptide effects on cAMP production and both protein phosphorylation and polyphosphoinositide metabolism seems probable in view of the importance of Ca^{2+} and anionic phospholipids (213). Such a direct action of the neuropeptides on the membrane may underlie secondary effects on neurotransmitter metabolism, protein synthesis, and other neurochemical parameters. A tentative working hypothesis has therefore been proposed. The neurochemical action of ACTH (and related peptides) may involve the following sequence of events (213). *1*) Neuropeptide binds to a membrane receptor consisting of a protein part and a lipid part. *2*) A change in the phosphorylation of a membrane enzyme occurs (a lipid kinase). *3*) Polyphosphoinositide formation is stimulated at the expense of PA. *4*) Several events consequently take place such as *a*) membrane hyperpolarization, *b*) decreased Ca^{2+} influx, *c*) decreased cAMP production, *d*) modulated action on classic neurotransmitters, and *e*) changes in protein synthesis.

XIII. CONCLUSION

The behavioral effects of ACTH have been studied for more than 25 years. The first report, by Mirsky et al. (30), suggested that ACTH reduced the effectiveness of an anxiety-producing stimulus. The finding that the behaviorally active moiety of ACTH resides in only a few amino acid residues and is independent of its peripheral endocrine effects came much later (483). At this time peptide chemistry allowed the synthesis of many different peptides in a relatively short period of time. During the last decade a great number of studies have attempted to elucidate the behavioral effect of ACTH and related peptides by the use of various behavioral paradigms. The influence of these peptides has been described in terms of diverse processes such

as reduced or increased fear, facilitated motivation and vigilance, enhanced concentration and (visual) attention, promoted learning, and stimulated memory-retrieval processes. Although this diversity seems to suggest that ACTH and related peptides have multiple behavioral effects, more probably there is one basic influence that is expressed differently, depending on the behavioral paradigm used. A basic effect of the peptide ACTH may therefore be reflected in effects on active or passive avoidance behavior, rewarded behavior, and social and sexual behavior. Another important factor contributes to the seemingly controversial data. The studies differ in a number of factors, such as the internal state of the subject, the strain and species of the animals used, the nature of the environment in which the animal is tested, the amount of peptide used, the route of administration, the experience of the investigator, and the correct replication of the experiments and the experimental procedure. For example, ACTH may enhance or attenuate retention of an inhibitory avoidance task depending on whether a low or a high dose is employed (291). Similarly the route of administration is of paramount importance, since ACTH induces excessive grooming after ICV but not after peripheral injection (140). The influence of neuropeptides on brain function should therefore be explored by the use of multiple doses at various time intervals; these peptides should be administered systemically as well as intracranially.

An important type of experiment is the structure-activity study in which the active site or sites of a neuropeptide for a given behavioral effect can be determined. Structure-activity studies have yielded a wealth of information about the effects of ACTH. The active core of ACTH for acquisition and extinction of active avoidance behavior appeared to be located in the sequence $ACTH_{4-7}$; other sites were also important (163). It was initially assumed that the active site for all behavioral effects of ACTH resided in the same amino acid sequence, but later research showed that this is not the case: one needs a bigger part of the ACTH molecule to induce excessive grooming (140). Moreover $ACTH_{1-24}$ and $ACTH_{4-10}$ facilitate the acquisition of a black-and-white discrimination problem, whereas α- and β-MSH (which contain the sequence $ACTH_{4-10}$) were inactive. Conversely, α- and β-MSH facilitated reversal learning of the same black-and-white discrimination problem, whereas $ACTH_{1-24}$ and $ACTH_{4-10}$ were inactive (385). It has been possible to potentiate the behavioral effect of $ACTH_{4-10}$ manyfold (162). The $ACTH_{4-9}$ analogue Org 2766 possessed 1,000-fold more activity on extinction of pole-jumping avoidance behavior, but it has lost nearly all its inherent melanotropic, fat-mobilizing, opiatelike, and corticotropic effects. One may ask whether the behavioral profile of Org 2766 is the same as that of $ACTH_{4-10}$. This question is particularly important, because potentiated compounds such as Org 2766 are tested for clinical use based on the profile established for the parent compound. There is some experimental evidence for effects of $ACTH_{4-10}$ on memory retrieval. However, neither $ACTH_{4-10}$ nor Org 2766 affect immediate or delayed memory, verbal or visual memory,

memory retrieval in healthy volunteers (93, 127, 303, 304, 456, 470), or memory in cognitively impaired elderly subjects (62, 114). Animal experiments pointed to an effect of ACTH on motivation (486), attention, and concentration (385). In fact $ACTH_{4-10}$ has been shown to enhance concentration and visual attention (129, 224, 385) and to improve task-oriented motivation in humans (127). No clear effects of $ACTH_{4-10}$ on mood have been detected in the human, but all studies employed single doses. Although Org 2766 also affected motivation in healthy volunteers (128), effects of this analogue after chronic administration appeared to be mainly on the mood of the subjects. In aged subjects it caused an increase in friendliness, a decrease in unsociability, a decrease in irritability, an increase in motivation, improved ward behavior, a reduction in self-rated anxiety and depression, and an increase in self-rated competence (345).

It is striking that different peptide structures within the pro-opiocortin molecule have opposite effects. We suggested in a previous review (143) that β-LPH may generate at least two classes of peptides with opposite effects on brain function (ACTH and MSH on the one hand and endorphins on the other). This hypothesis may now be extended to the pro-opiocortin molecule. For example, β-endorphin and related endorphins induce analgesia (53), catatonia (34), or catalepsy (205); attenuate sexual behavior (299); bind to opiate receptors in the brain (56); inhibit firing rate in CNS areas rich in opiate receptors (320); reduce catecholamine and acetylcholine transmission (462); and reduce cAMP activity (498). Conversely ACTH, MSH, and related peptides counteract morphine-induced analgesia (139), morphine-induced excitation (226), attenuate β-endorphin-induced catatonia (353), reduce tonic immobility (425), facilitate sexual motivation (38), bind to opiate-receptor sites (437), compete with β-endorphin for opiate-receptor sites (2), increase firing rate (134), attenuate auditory evoked potentials after ICV-administered β-endorphin, block epileptogenic spiking in the area of the nucleus gigantocellularis after microinjection into the periaqueductal gray (385), antagonize opiatelike effects on spinal reflex activity (528), increase catecholamine and acetylcholine transmission (462), and stimulate cAMP activity in brain slices (498). Administration of γ_2-MSH also affects extinction of pole-jumping avoidance behavior in a manner opposite to that of β-endorphin. The ACTH-like peptides and β-endorphin do differ in that ACTH induces stretching and yawning in the rat but β-endorphin does not. This later peptide, however, induces excitation, as manifested by quick movements of body and head, jumping, gnawing, and body shakes (140). Some behavioral effects of ACTH and β-endorphin, however, are similar. They have similar effects on aversively motivated behavior (491), although amnesic effects of β-endorphin also have been reported (200). Both induce excessive grooming behavior: this grooming behavior induced by these two classes of peptides differs in composition (140). Finally, ACTH and related peptides and β-endorphin affect hippocampal slow activity in the same direction (453, 491). Despite the fact that ACTH and β-endorphin have similar effects on those

three parameters, the evidence for opposite effects of ACTH- and MSH-like peptides and β-endorphin is impressive. If the biotransformation of pro-opiocortin generates neuropeptides with inhibitory and excitatory effects in the CNS, this mechanism may have a profound effect on brain homeostasis. Some evidence favoring this notion has been obtained. Adrenocorticotropin elicited a morphinelike abstinence syndrome after microinjection into the periaqueductal gray (203). It was suggested that opiate effects are mediated by two receptors: a stereospecific opiate β-endorphin receptor that mediates the analgesic and cataleptic effects and a nonstereospecific opiate ACTH receptor that mediates the opiate-abstinence syndrome and the excitatory effects of the opiates. In this respect γ_2-MSH is more sensitive than ACTH (353). This hypothesis could explain the opposite effects of ACTH-like peptides and β-endorphin. Another possibility is that the opposite effects of ACTH and β-endorphin are caused by competition of these peptides at the level of the receptor (437, 472) or by a functional antagonism as has been suggested for γ_2-MSH.

An interaction between β-endorphin and other brain peptides has also been demonstrated. Vasopressin and oxytocin affected the development of tolerance to and physical dependence on opioids, and these neurohypophyseal hormones may modulate the effects of the endorphins (354). A general conclusion may be that the various neuropeptides with behavioral effects opposite to those of β-endorphin compete with the endorphins for peptide-sensitive structures in the brain. Information processing by the brain is thereby altered, eventually allowing the organism to interact more efficiently with its environment.

The peptides related to β-endorphin induce a variety of behavioral effects. Apart from the fact that β-endorphin caused analgesia and catatonia/catalepsy, this peptide appeared to be a second-order precursor molecule for neuropeptides with neuroleptic and psychostimulant effects. The brain is capable of transforming the molecule into neuropeptides with such effects. This is performed by proteolytic enzymes that cleave the 77–78 bond of β-endorphin to form γ-endorphin and subsequently release the amino acid tyrosine in position 61. The resulting des-tyrosine analogue DTγE (67) is probably converted to β-LPH_{66-77} (DEγE) by the removal of the remaining four amino acids of Met-enkephalin. Both DTγE and DEγE had neuroleptic activity (494). Interestingly such a biotransformation of an opiatelike to a neurolepticlike compound is reminiscent of the fact that an important class of neuroleptic drugs, the butyrophenones, were originally developed from the synthetic opiate pethidine (210).

Both DTγE and DEγE facilitated extinction of active avoidance behavior, attenuated passive avoidance behavior, induced positive grasping responses that may be regarded as a physiological form of catalepsy, and reduced electrical self-stimulation from the ventral tegmental area and the nucleus accumbens (355). Somewhat similar effects were found after treatment with neuroleptic drugs such as haloperidol. The γ-type endorphins,

however, lack the sedation and the decrease in locomotor activity characteristic of neuroleptic drugs. In addition DTγE does not displace the binding of [^{3}H]spiperone, [^{3}H]haloperidol, or [^{3}H]apomorphine from their specific binding sites in rat brain membrane preparations in vitro (341, 349), and therefore cannot be regarded as a classic neuroleptic compound. Nevertheless clinical studies clearly demonstrated antipsychotic effects of DTγE (460). Animal experiments with the γ-type endorphins indicate that these peptides lack an influence on the extrapyramidal and autonomic nervous system, whereas neurological disorders of the extrapyramidal system and increased autonomic nervous system activity are characteristic effects of neuroleptic drugs. The γ-type-endorphins may therefore be more specific than classic neuroleptics. They may affect transmitter activity of those structures in the brain involved in certain types of psychopathology. For instance, mesolimbic DA pathways may be involved in the pathogenesis of schizophrenia. These pathways are also affected by γ-type endorphins (141). Studies by van Ree et al. (360) indicate that DEγE may influence dopaminergic systems indirectly. The mechanism involved may possibly be a modulation of presynaptically located DA receptors. These are preferentially activated by low doses of apomorphine, resulting in decreased ambulation. This effect was dose-dependently antagonized by DEγE (360). This suggested that DEγE resembles a DA antagonist that acts exclusively on self-inhibiting DA receptors. Studies by Nickolson et al. (318, 393) point to a similar mode of action. Classic neuroleptics, in contrast, act additionally via binding to postsynaptic DA receptors that become supersensitive on chronic treatment. Thus the occurrence of extrapyramidal symptoms after treatment with classic neuroleptics and the lack of such side effects of γ-type endorphins may be explained by a differential effect of these substances on the two types of DA receptors.

Another important observation was made, showing that α-endorphins have behavioral effects opposite to those of the γ-type endorphins. The effects of α-endorphin and DTαE resemble in certain aspects those of amphetamine in animal experiments, except for the stimulatory influence of amphetamine on locomotor activity and the induction of stereotypy. This finding and the observations on the neuroleptic and the antipsychotic effects of γ-type endorphins formed the basis of the hypothesis that an inborn error in the generation or metabolism of DTγE or DEγE is an etiological factor in schizophrenia (493). This hypothesis assumes that the formation of DTγE or DEγE is the rate-limiting step in the conversion of β-endorphin. A deficiency in these compounds may therefore lead to a derangement in β-endorphin homeostasis. When the feedback regulation is disturbed, excess β-endorphin will accumulate in the brain and may lead to catatonic schizophrenia. This prediction is supported by animal experiments in which excess β-endorphin in the brain has been shown to induce catatonia (34). Other derangements of β-endorphin metabolism may also be possible: excess amounts of α-endorphin might be generated due to errors in the biotransformation of β-endorphin or as a result of alterations in proteolytic enzyme activity. This

latter possibility seems probable in view of recent findings that slight alterations in pH dramatically alter the activity of enzymes involved in the generation of α- versus γ-type endorphins in brain membranes (67). Endogenously released α-endorphin may induce effects that in some respects are similar to those of amphetamines. These drugs have been shown to mimic the aggressive or paranoid form of the schizophrenic syndrome (296). Other disturbances in β-endorphin biotransformation are feasible, but the main disturbance in schizophrenia in this view would be caused by an imbalance between α- and γ-type endorphins. The data from animal experiments suggest that changes in the metabolism of the endorphins could underlie the behavioral and other disturbances observed in certain types of psychopathology in humans. Proof for this is not yet available, but an important implication of this tentative hypothesis is that such disturbances in neuroendocrine regulation may eventually be corrected by administration of the deficient neuroendocrine principle.

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